Table 20: **Nef**

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(13–20)	Nef(13–20 LAI)	WPTVRERM	HIV-1 infection	human(B*0801)	[Brander & Goulder(2001), Goulder (1997g)]
	• C. Brander notes this is a B*0801 epitope				
Nef(13–20)	Nef(13–20 LAI)	WPTVRERM	HIV-1 infection	human(B8)	[Goulder (1997g)]
	• Unusual epitope for HLA-B8, but compatible with crystal structure predictions				
Nef(13–20)	Nef(13–20)	WPTVRERM	HIV-1 infection	human(B8)	[Betts (2000)]
	• Only 4/11 HLA-A2+ HIV+ individuals had CTL that reacted to SLYNTVATL, calling into question whether it is immunodominant • Ninety-five optimally-defined peptides from this database were used to screen for γ interferon responses to other epitopes • 1/11 of the HLA A2+ was HLA A*0201, A31, B8, B51 and responded to this epitope as well as seven others				
Nef(13–20)	Nef(13–20 SF2)	WPTVRERM	HIV-1 infection	human(B8)	[Altfeld (2001c)]
	• Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection • The breadth and specificity of the response was determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef • Previously described and newly-defined optimal epitopes were tested for CTL response • Number of HLA-B8+ individuals that had a CTL response to this epitope broken down by group: 1/3 group 1, 0/3 group 2, and 1/2 group 3				
Nef(13–20)	Nef(13–20)	WPTVRERM	HIV-1 infection	human(B8)	[Day (2001)]
	• B8-restricted CTL accounted for about 1/3 of the total CTL response in one individual				
Nef(42–50)	Nef(44–52 HXB3)	ALTSSNTAA	Vaccine	murine(HLA-A201 transgenic)	[Sandberg (2000)]
	Vaccine: *Vector/type:* DNA, peptide *Strain:* HXB3 *HIV component:* Nef *Stimulatory Agents:* Freund's adjuvant • Ten Nef 9-mer peptides were predicted to have a strong binding affinity with HLA-A*0201 – of these, four did bind strongly by a T2 class I stabilization assay, several others bound weakly • A CTL immune response to only 3/10 peptides was detected by a 51Cr-release assay after immunization of HLA-A201 transgenic mice with either nef DNA under the control of a CMV promotor, coated on gold particles delivered to abdominal skin by gene gun • ALTSSNTAA was also tested by subcutaneous injection of Nef peptides in Freund's adjuvant • ALTSSNTAA bound weakly to HLA-A2, but it had the strongest CTL response among the three elicited by the DNA vaccine and a strong response to the peptide vaccination				

Nef(62–81)	Nef(61–80)	EEEEVGFPVTPQVPLR-PMTY	*in vitro* stimulation	human( )	[Lieberman (1995)]

- HIV-specific CTL lines developed by *ex vivo* stimulation with peptide

Nef(62–81)	Nef(61–80 SF2)	EEEEVGFPVTPQVPLR-PMTY	HIV-1 infection	human( )	[Lieberman (1997a)]

- Of 25 patients, most had CTL specific for more than one HIV-1 protein
- Twelve subjects had CTL that could recognize vaccinia-expressed LAI Nef
- Two of these 12 had CTL response to this peptide
- The responding subjects were HLA-A11, A24, B8, B35, and HLA not determined

Nef(62–81)	Nef(61–80 SF2)	EEEEVGFVTPQVPLRP-MTY	HIV-1 infection	human( )	[Lieberman (1997b)]

- CTL expanded *ex vivo* were later infused into HIV-1 infected patients

Nef(62–81)	Nef( )	EEEEVGFPVTPQVPLR-PMTY	HIV-1 infection	human( )	[Altfeld (2001a)]

- HIV+ individual AC-06 was tested for reactive overlapping peptides spanning all HIV-1 proteins in an ELISPOT and was found to react with 12 peptides from 7 proteins, suggesting that the breadth of CTL responses is underestimated if accessory proteins are not included in the study
- Nef peptides PQVPLRRMTYKAAVDLSHFL, KAAVDLSHFLKEKGGLEGLI and EEEEVGFPVTPQVPLRPMTY were recognized and the first two share KAAVDLSHFL (a Cw8 epitope), the first and last share PQVPLRPMTY

Nef(66–80)	Nef(66–80 BRU)	VGFPVTPQVPLRMT	HIV-1 infection	human(A1, B8)	[Hadida (1992)]

- HIV-1 specific CTLs detected in lymphoid organs of HIV-1 infected patients

Nef(66–80)	Nef(64–78)	VGFPVTPQVPLRMT	HIV-1 infection	human(A1, B8)	[Ferrari (2000)]

- One of the 51 HIV-1 epitopes selected by Ferrari *et al*. as good candidate CTL epitopes for vaccines by virtue of being conserved and presented by common HLA alleles

Nef(66–97)	Nef(66–97 LAI)	VGFPVTPQVPLRPMT-YKAAVDLSHFLKEKGG-L	Vaccine	human( )	[Gahery-Segard (2000)]

Vaccine: *Vector/type:* lipopeptide *HIV component:* six peptides

- Anti-HIV lipopeptide vaccine consisting of six long amino acid peptides derived from Nef, Gag and Env HIV-1 proteins modified by a palmitoyl chain was administered in a phase I trial
- A CD4+ T-cell proliferative response to at least one of the six peptides was observed in 9/10 vaccinees – 5/10 reacted to this Nef peptide
- 9/12 tested mounted a CTL response to at least one of the six peptides; each of the six peptides elicited a CTL response in at least one individual

	• 5/12 tested had an IgG response to this peptide				
Nef(68–76)	Nef(72–80 SF2)	FPVRPQVPL	HIV-1 infection	human(B*3501)	[Tomiyama (1997)]
	• A CTL clone responsive to this epitope was obtained • 3/7 B35-positive individuals had a CTL response to this epitope • An R to T substitution at position 4 abrogates specific lysis, but not binding to B*3501				
Nef(68–76)	Nef(72–80)	FPVRPQVPL	HIV-1 infection	human(B*3501)	[Tomiyama (2000a)]
	• CD8+ T-cells that bound one of six HIV-specific B*3501-epitope tetramers did not express CD28 or CD45A • A significant increase in CD28-CD45RA- cells and a decrease of CD28+CD45RA+ cells was observed in chronically HIV-1-infected individuals relative to healthy individuals • CD28-CD45RA- cells are likely to be effector cells and have high levels of perforin in their cytoplasm • The mean percentage of total CD28- CD8+ cells in chronically infected HIV-1-infected patients was 76.6% in comparison to HIV-1-uninfected individuals (40.6%)				
Nef(68–76)	Nef(72–80 SF2)	FPVRPQVPL	HIV-1 infection	human(B*3501)	[Shiga (1996)]
	• Binds HLA-B*3501				
Nef(68–76)	( )	FPVRPQVPL	HIV-1 infection	human(B35)	[Kawana (1999)]
	• HLA B35 is associated with rapid disease progression • The sequences of 9 previously described HIV-1 B35 CTL epitopes were obtained in 10 HLA B35+ and 19 HLA B35- individuals • 3/9 CTL epitopes had substitutions that were more common in B35+ individuals than in B35- individuals, but this was one of the six that had no B35 associated pattern of mutation				
Nef(68–76)	Nef(66–74)	FPVRPQVPL	HIV-1 infection	human(B35)	[Ferrari (2000)]
	• One of the 51 HIV-1 epitopes selected by Ferrari *et al.* as good candidate CTL epitopes for vaccines by virtue of being conserved and presented by common HLA alleles				
Nef(68–76)	Nef(68–76)	FPVTPQVPL	*in vitro* stimulation	human(B7)	[Wilson (1999b)]
	• Dendritic cells are the most potent for priming T-cell responses – DCs can stimulate autologous CTL responses from T-cells cultured from HIV negative donors • Th1-biasing cytokines IL-12 or IFNα enhance CTL responses *in vitro* whether the epitope is delivered by pulsing from peptide, or expressed from within • B7 and A2 Nef epitopes were studied – FPVTPQVPL has a high affinity for B7				
Nef(68–76)	Nef(68–76)	FPVTPQVPL	HIV-1 infection	human(B7)	[Day (2001)]
	• The CTL response to optimally defined CTL epitopes restricted by HLA class I A and B alleles in individuals who co-expressed HLA A2, A3, and B7 was studied in eight HIV-1-infected subjects, two with acute infection, five with chronic, and one long-term non-progressor (LTNP) • Two to 17 epitopes were recognized in a given individual, A2-restricted CTL response tended to be narrow and never dominated the response, and 25/27 epitopes were targeted by at least one person				

- Subjects with chronic HIV-1 infection recognized between 2-8 out of 11 B7-restricted epitopes
- An acute seroconvertor homozygous for the B7 allele recognized five B7-restricted epitopes
- The other acute seroconvertor failed to recognize any of the 11 B7-restricted epitopes tested
- The B7-restricted CTL response was highly variable and there was no clearly dominant epitope

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Nef(68–77) Nef(68–77 LAI) FPVTPQVPLR HIV-1 infection human(B*0702) [Brander & Goulder(2001)]

- C. Brander notes this is a B*0702 epitope

Nef(68–77) Nef(68–77 LAI) FPVTPQVPLR HIV-1 infection human(B7) [Haas (1996)]

- There was a high degree of variation in three CTL epitopes in Nef in four slow and non-progressors, and variant specific CTLs arose over time to eliminate variants, indicating immune selection

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Nef(68–77) Nef( ) FPVTPQVPLR HIV-1 infection human(B7) [Kaul (2001b)]

- This study examines CTL responses in HIV-exposed, persistently seronegative individuals, HEPS, who eventually seroconverted – 11/114 HEPS Nairobi sex workers eventually seroconverted, and for six of these HIV CTL reactive epitopes had been defined while seronegative
- FPVTPQVPLR was recognized in 1 of the 6 women (ML1203), and the response was present in the last available sample prior to seroconversion, 7 months
- 20/20 sequences of the infecting strain had no substitutions in this epitope, all were FPVTPQVPLR, so there was no evidence for escape
- The epidemiological factor associated with seroconversion was stopping sex work and HIV-specific CTL activity declines when HEPS sex workers stop working for a period or retire
- This epitope was recognized in 1/22 HEPS sex worker controls, ML851

Nef(68–77) Nef(66–75) FPVRPQVPLR HIV-1 infection human(B7) [Ferrari (2000)]

- One of the 51 HIV-1 epitopes selected by Ferrari *et al.* as good candidate CTL epitopes for vaccines by virtue of being conserved and presented by common HLA alleles

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Nef(68–77) Nef(68–77 SF2) FPVTPQVPLR HIV-1 infection human(B7) [Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection
- The breadth and specificity of the response was determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response
- Number of HLA-B7+ individuals that had a CTL response to this epitope broken down by group: 0/4 group 1, 0/3 group 2, and 1/1 group 3

Nef(68–77) Nef(68–77) FPVTPQVPLR HIV-1 exposed seronegative, HIV-1 infection human(B7) [Kaul (2001a)]

- ELISPOT was used to study CTL responses to a panel of 54 predefined HIV-1 epitopes in 91 HIV-1-exposed, persistently seronegative (HEPS) and 87 HIV-1-infected female Nairobi sex workers
- Responses in HEPS women tended to be lower, and focused on different epitopes with HLA presenting molecules that have previously been associated with reduced risk of infection, and there was a shift in the response in the HEPS women upon late seroconversion to epitopes recognized by the HIV-1-infected women
- 43/91 HEPS women had CD8+ responses and detection of HIV-1-specific CTL in HEPS women increased with the duration of viral exposure
- Subject ML 1203 started with CTL responses to A*6802 DTVLEDINL and to B7 FPVTPQVPLR prior to seroconversion, and upon seroconversion acquired additional responses to A*6802 ETAYFILKL which became dominant, B7 TPGPGV/IRYPL, B7 IPRRIRQGL, and B7 SPRTLNAWV

Nef(68–77)	Nef(68–77)	FPVTPQVPLR	HIV-1 infection	human(B7)	[Day (2001)]

- The CTL response to optimally defined CTL epitopes restricted by HLA class I A and B alleles in individuals who co-expressed HLA A2, A3, and B7 was studied in eight HIV-1-infected subjects, two with acute infection, five with chronic, and one long-term non-progressor (LTNP)
- Two to 17 epitopes were recognized in a given individual, A2-restricted CTL response tended to be narrow and never dominated the response, and 25/27 epitopes were targeted by at least one person
- Subjects with chronic HIV-1 infection recognized between 2-8 out of 11 B7-restricted epitopes
- An acute seroconvertor homozygous for the B7 allele recognized five B7-restricted epitopes
- The other acute seroconvertor failed to recognize any of the 11 B7-restricted epitopes tested
- The B7-restricted CTL response was highly variable and there was no clearly dominant epitope

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Nef(68–84)	Nef( )	FPVRPQVPLRPMTYK-GA		human( )	[Jubier-Maurin (1999)]

- 41 new HIV-1 strains describing envelope subtypes of HIV-1 A-H were genetically characterized in the nef coding region – 34 subtypes were classified in the same subtype in nef and env and 7 of the 41 strains were recombinants
- This region was defined as a CTL epitope region that is conserved among HIV-1 M group subtypes

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Nef(69–79)	( )	RPQVPLRPMTY	HIV-1 infection	human(B35)	[Kawana (1999)]

- HLA B35 is associated with rapid disease progression
- The sequences of 9 previously described HIV-1 B35 CTL epitopes were obtained in 10 HLA B35+ and 19 HLA B35- individuals
- 3/9 CTL epitopes had substitutions that were more common in B35+ individuals than in B35- individuals – only one of these reduced the binding of the peptide to B35 and was shown to be an escape mutation
- ----------F was found in 9/10 of the B35+ individuals, none of the B35- individuals – the Y –> F substituted peptide had a similar binding affinity with B35 and was recognized by a CTL clone equally with wildtype

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Nef(71–79)	Nef(71–79 LAI)	TPQVPLRPM	HIV-1 infection	human(B*0702)	[Brander & Goulder(2001)]

- C. Brander notes this is a B*0702 epitope

Nef(71–79)	Nef(71–79 SF2)	TPQVPLRPM	HIV-1 infection	human(B7)	[Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection
- The breadth and specificity of the response was determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response
- Number of HLA-B7+ individuals that had a CTL response to this epitope broken down by group: 0/4 group 1, 0/3 group 2, and 1/1 group 3

Nef(71–79)	Nef(71–79)	TPQVPLRPM	HIV-1 infection	human(B7)	[Day (2001)]

- The CTL response to optimally defined CTL epitopes restricted by HLA class I A and B alleles in individuals who co-expressed HLA A2, A3, and B7 was studied in eight HIV-1-infected subjects, two with acute infection, five with chronic, and one long-term non-progressor (LTNP)
- Two to 17 epitopes were recognized in a given individual, A2-restricted CTL response tended to be narrow and never dominated the response, and 25/27 epitopes were targeted by at least one person
- Subjects with chronic HIV-1 infection recognized between 2-8 out of 11 B7-restricted epitopes
- An acute seroconvertor homozygous for the B7 allele recognized five B7-restricted epitopes
- The other acute seroconvertor failed to recognize any of the 11 B7-restricted epitopes tested
- The B7-restricted CTL response was highly variable and there was no clearly dominant epitope

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Nef(71–81)	Nef(75–85 SF2)	RPQVPLRPMTY	HIV-1 infection	human(B*3501)	[Tomiyama (1997)]

- A CTL clone responsive to this epitope was obtained
- 4/7 B35-positive individuals had a strong CTL response to this epitope
- An R to T substitution at position 1 abrogates specific lysis, but not binding to B*3501
- An R to H substitution at position 7 did not alter reactivity

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Nef(71–81)	Nef(75–85)	RPQVPLRPMTY	HIV-1 infection	human(B*3501)	[Tomiyama (2000a)]

- CD8+ T-cells that bound one of six HIV-specific B*3501-epitope tetramers did not express CD28 or CD45A
- A significant increase in CD28-CD45RA- cells and a decrease of CD28+CD45RA+ cells was observed in chronically HIV-1-infected individuals relative to healthy individuals
- CD28-CD45RA- cells are likely to be effector cells and have high levels of perforin in their cytoplasm
- The mean percentage of total CD28- CD8+ cells in chronically infected HIV-1-infected patients was 76.6% in comparison to HIV-1-uninfected individuals (40.6%)

Nef(71–81)	Nef(75–85 SF2)	RPQVPLRPMTY	HIV-1 infection	human(B*3501)	[Shiga (1996)]

- Binds HLA-B*3501

Nef(71–81)	Nef(69–79)	RPQVPLRPMTY	HIV-1 infection	human(B35)	[Ferrari (2000)]

- One of the 51 HIV-1 epitopes selected by Ferrari *et al*. as good candidate CTL epitopes for vaccines by virtue of being conserved and presented by common HLA alleles

Nef(72–79)	Nef( )	VPLRPMTY	HIV-1 exposed seronegative	human(B35)	[Kaul (2000)]

- 11/16 heavily HIV exposed but persistently seronegative sex-workers in Nairobi had HIV-specific CD8 γ-IFN responses in the cervix – systemic CD8+ T-cell responses tended to be to the same epitopes but at generally lower levels than cervical CD8+ T-cell responses
- Low risk individuals did not have such CD8+ cells
- CD8+ T-cell epitopes DTVLEDINL (3 individuals), SLYNVATL (4 individuals), LSPRTLNAW (3 individuals) and YPLTFGWCF (4 individuals) were most commonly recognized by the HIV-resistant women

Nef(72–79)	Nef( )	VPLRPMTY	HIV-1 infection	human(B35)	[Wilson (2000)]

- Three individuals with highly focused HIV-specific CTL responses were studied during acute infection using tetramers – high frequencies of HIV-1-specific CD8+ T-cells were found prior to seroconversion, and a close temporal relationship between the number of circulating HIV-specific T-cells and viral load was also found
- All three patients were B*2705, with HLA alleles: A1, A30/31, B*2705, B35; A1, A*0301, B7, B*2705; and A*0201, A*0301, B*2705, B39
- ELISPOT was used to test a panel of CTL epitopes that had been defined earlier and was appropriate for the HLA haplotypes of the study subjects – 3/3 subjects showed a dominant response to the B*2705 epitope KRWIILGGLNK
- The subject with A*0201 had a moderately strong response to SLYNTVATL
- Weak responses were observed to A*301-RLRPGGKKK, A*301-QVPLRPMTYK, and B7-TPGPGVRYPL in the subject who was HLA A1, A*0301, B7, B*2705
- No acute response was detected to the following epitopes: A*201-ILKEPVHGV, A*301-KIRLRPGGK, A*301-AIFQSSMTK, A*301-TVYYGVPVWK, B35-EPIVGAETF, B35-HPDIVIYQY, B35-PPIPVGEIY, B35-NSSKVSQNY, B35-VPLRPMTY, B35-DPNPQEVVL

Nef(72–91)	Nef(71–90 SF2)	PQVPLRMTYKAAVDL-SHFL	HIV-1 infection	human( )	[Lieberman (1997a)]

- Of 25 patients, most had CTL specific for more than one HIV-1 protein
- Eleven subjects had CTL that could recognize vaccinia-expressed LAI Nef
- Three of these 11 had CTL response to this peptide
- The responding subjects were HLA-A3, A32, B51, B62; HLA-A11, A24, B8, B53

Nef(72–91)	Nef(71–90 SF2)	PQVPLRPMTYKAAVD-LSHFL	HIV-1 infection	human( )	[Lieberman (1997b)]

- CTL expanded *ex vivo* were later infused into HIV-1 infected patients

Nef(72–91)	Nef( )	PQVPLRRMTYKAAVD-LSHFL	HIV-1 infection	human( )	[Altfeld (2001a)]

- HIV+ individual AC-06 was tested for reactive overlapping peptides spanning all HIV-1 proteins in an ELISPOT and was found to react with 12 peptides from 7 proteins, suggesting that the breadth of CTL responses is underestimated if accessory proteins are not included in the study
- Nef peptides PQVPLRRMTYKAAVDLSHFL, KAAVDLSHFLKEKGGLEGLI and EEEEVGFPVTPQVPLRPMTY were recognized and the first two share KAAVDLSHFL (a Cw8 epitope), the first and last share PQVPLRPMTY

Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV-1 infection	human( )	[Garcia (1997)]

- The anti-Nef CTL line P1 specific for this epitope is able to kill target cells via two mechanisms
- First: Ca^{2+}-dependent, perforin-dependent Nef-specific lysis
- Second: Ca^{2+}-independent, CD95-dependent apoptosis that could also kill non-specific targets
- Findings indicate that the two mechanisms are not mutually exclusive in human CTL, as they are in mice
- CTL mediated CD95-dependent apoptosis may play a role in pathogenesis

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Nef(73–82)	Nef(73–82 NL43)	QVPLRPMTYK	HIV-1 infection	human(A*0301)	[Koenig (1990)]

- 81 Tyr is critical for binding to A3.1
- C. Brander notes that this is an A*0301 epitope in the 1999 database

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Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK		human(A*0301)	[Brander & Goulder(2001)]

- C. Brander notes this is an A*0301 epitope

Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV-1 infection	human(A11)	[Le Borgne (2000)]

- Soluble factors in supernatant from both an HIV-specific cloned CTL line and an EBV (Epstein-Barr-virus) CTL line inhibit viral replication, but do not block viral entry in CD4+ T lymphocytes, by a noncytotoxic mechanism

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Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK	HIV-1 infection	human(A11)	[Robertson (1993)]

- Development of a retroviral vector (pNeoNef) to generate autologous CTL targets
- [Hunziker1998] suggests that HLA-A2 does not in fact present this epitope
- The initial assignment of HLA-A2 presentation for this epitope was based on a serological HLA typing. Subsequently, the authors revisited the issue with genetic HLA typing and found that HLA-A11 was the correct presenting molecule (Dr. Florence Buseyne, Pers. Comm., 2000)

Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK	HIV-1 infection	human(A11)	[Couillin (1994), Goulder (1997a)]

- Mutational variation in HIV epitopes in individuals with appropriate HLA types can result in evasion of CTL response
- [Goulder (1997a)] is a review of immune escape that summarizes this study

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Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK	HIV-1 infection	human(A11)	[Couillin (1995)]

- Mutations found in this epitope in HLA-A11 positive and negative donors were characterized

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Nef(73–82)	( )	QVPLRPMTYK		(A11)	[Brander & Goulder(2001), Buseyne(1999)]

Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV-1 infection	human(A11)	[Oxenius (2000)]

- Epitope name: QVP. Patients who started therapy at acute HIV-1 infection (three with sustained therapy, two with limited therapy upon early infection) had strong HIV-specific CD4 proliferative responses and were able to maintain a CTL response even with undetectable viral load – three patients that had delayed initiation of HAART had no HIV-specific CD4 proliferative responses and lost their CTL responses when HAART was eventually given and their viral loads became undetectable
- One of the 2/8 HLA-A11 study subjects recognized this CTL epitope
- Patient SC18(HLA A2/11, B8/44, Cw06/0701, DR3/7, DR52/53, DQ2) recognizes the epitopes ACQGVGGPGHK, QVPLRPMTYK, AVDLSHFLK, and one called QIY but not fully described – he had brief therapy upon seroconversion and has had low viral load during 600 days of follow up

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Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV-1 exposed seronegative, HIV-1 infection	human(A11)	[Kaul (2001a)]

- ELISPOT was used to study CTL responses to a panel of 54 predefined HIV-1 epitopes in 91 HIV-1-exposed, persistently seronegative (HEPS) and 87 HIV-1-infected female Nairobi sex workers

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Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV-1 infection	human(A11)	[Appay (2000)]

- Combined tetramer and intracellular cytokine staining was used to study the function of circulating CD8+ T-cells specific for HIV and CMV
- HIV-specific CD8+ T-cells expressed lower levels of perforin than CMV-specific CD8+ T-cells from the same donor, and this was associated with persistent CD27 expression on HIV-specific cells, suggesting impaired maturation
- In most donors, between 50% and 95% of the activated virus-specific CD8+ T-cells produced IFN-γ and MIP-1β with a distinct subset that failed to produce TNF-α

Nef(73–82)	Nef(71–80 93TH253 CRF01)	QVPLRPMTYK	HIV-1 exposed seronegative, HIV-1 infection	human(A11)	[Sriwanthana (2001)]

- Epitope name: N73-82. This was a study of HIV-1 exposed persistently seronegative (HEPS) female sex workers in Chiang Mai, northern Thailand
- HLA-A11 is very common in this population, and was enriched among the HEPS sex workers – weak CTL responses were detected in 4/7 HEPS women, and CTL responses were found in 8/8 HIV+ controls, and 0/9 HIV- women that were not exposed
- This epitope was weakly reactive in HEPS study subjects 265 who was HLA A2/A11 and 128 who was HLA A11/A33, and after a second *in vitro* stimulation, in study subject 256 who was HLA A11/33, making it the most reactive epitope tested in HLA-A11 HEPS women, with 3/4 responding
- This epitope was strongly reactive in HIV+ study subject 053 who carried HLA-A11

Nef(73–82)	Nef(71–80 93TH253 CRF01)	QVPLRPMTYK	HIV-1 infection	human(A11)	[Bond (2001)]

- HLA-A11 CRF01 (called subtype E in Bond *et al.*) epitopes were identified that stimulated CTL from HIV+ female sex workers (FSW) from Northern Thailand, of whom more than half were HLA-A11 positive
- 77 possible HLA-A11 epitopes were first defined using EpiMatrix, these were screened for binding to A11 and 26 bound, and 12 of these were epitopes for CTL responses from 8 HLA-A11 positive FSWs, six were novel, six were previously identified

- This epitope was predicted by the EpiMatrix method to be likely to bind to A11, and it served as an epitope in the FSWs, it was one of the six A11 epitopes that had been previously defined
- 4/8 tested FSWs recognized this epitope
- An HLA-A11 tetramer was made for this epitope, which was recognized by two subjects – only one subject had an expanded tetramer staining T-cell population after *in vitro* stimulation
- This epitope was highly conserved in other subtypes, and exact matches were common

Nef(73–82)	Nef(73–81)	QVPLRPMTYK	HIV-1 infection	human(A2, A3, A11, B35)	[Ferrari (2000)]

- One of the 51 HIV-1 epitopes selected by Ferrari *et al*. as good candidate CTL epitopes for vaccines by virtue of being conserved and presented by common HLA alleles

Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK	HIV-1 infection	human(A3)	[Chassin (1999)]

- Mutations in Nef that flank this epitope, Thr71Lys and Ala83Gly, may account for an observed loss of CTL reactivity, with escape due to the introduction of proteasome processing reduction

Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV-1 infection	human(A3)	[Durali (1998)]

- Cross-clade CTL response was studied by determining the CTL activity in seven patients from Bangui, (6 A subtype, and 1 AG recombinant infections) and one A subtype infection from a person living in France originally from Togo, to different antigens expressed in vaccinia
- Pol reactivity: 8/8 had CTL to A subtype, and 7/8 to B subtype, and HIV-2 Pol was not tested
- Gag reactivity: 7/8 reacted with A or B subtype gag, 3/8 with HIV-2 Gag
- Nef reactivity: 7/8 reacted with A subtype, and 5/8 with B subtype, none with HIV-2 Nef
- Env reactivity: 3/8 reacted with A subtype, 1/8 with B subtype, none with HIV-2 Env
- One of the patients was shown to react to this epitope: QVPLRPMTYK

Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK	HIV-1 infection	human(A3)	[Goulder (1997e), Goulder (1997a)]

- Identical twin hemophiliac brothers were both infected with the same batch of factor VIII
- Both had a response to this epitope
- [Goulder (1997a)] is a review of immune escape that summarizes this study

Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV-1 infection	human(A3)	[Lubaki (1997)]

- Eighty-two HIV-1-specific CTL clones from 5 long-term non-progressors were isolated and analyzed for breadth of response
- A sustained Gag, Env and Nef response was observed, and clones were restricted by multiple HLA epitopes, indicating a polyclonal response
- An A3+ subject had a strong response to this epitope, with 10/11 CTL clones being specific for this epitope, isolated at two time points, 1 year apart

Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV-1 infection	human(A3)	[Samri (2000)]

- Epitope name: N1. The epitope was recognized by patients 252#0 and 252#4 in a study of the effects of therapy escape mutations on CTL recognition

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Nef(73–82)	Nef(73–82 SF2)	QVPLRRMTYK	HIV-1 infection	human(A3)	[Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection
- The breadth and specificity of the response was determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response
- Number of HLA-A3+ individuals that had a CTL response to this epitope broken down by group: 3/7 group 1, 1/4 group 2, and 1/2 group 3

Nef(73–82)	Nef(73–82)	RLRDLLLIVTR	HIV-1 infection	human(A3)	[Day (2001)]

- The CTL response to optimally defined CTL epitopes restricted by HLA class I A and B alleles in individuals who co-expressed HLA A2, A3, and B7 was studied in eight HIV-1-infected subjects, two with acute infection, five with chronic, and one long-term non-progressor (LTNP)
- Two to 17 epitopes were recognized in a given individual, A2-restricted CTL response tended to be narrow and never dominated the response, and 25/27 epitopes were targeted by at least one person
- All patients recognized at least 1 A3 epitope, up to 8 A3 epitopes, but none was clearly dominant
- In two of the subjects, RLRDLLLIVTR was the dominant epitope

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Nef(73–82)	Nef( )	QVPLRPMTYK	HIV-1 infection	human(A3)	[Altfeld (2000)]

- This epitope was mapped by ELISPOT in a study identifying new HLA-B60 epitopes, and was one of the epitopes presented by a molecule other than B60 in an HLA-B60 individual

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Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK	HIV-1 infection	human(A3 supertype)	[Mollet (2000)]

- Epitope name: N1. A panel of 16 epitopes covering 15 class I alleles was tested in 14 HIV+ patients from an unselected Caucasian population treated with HAART, using CD8+ cell IFNγ production to measure responses
- In general, during the first month of treatment viral load decreased and frequencies of HIV-specific CTL tripled and broadened – eight new HIV specificities that were not previously detectable were newly detected, as were CMV specific CD8+ PBL – but with continued viral suppression, HIV-specific responses diminished
- Viral rebounds gave different patterns of response: increases or decreases in pre-existing response, new specificities, or no change

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Nef(73–82)	Nef(94–103)	QVPLRPMTYK	HIV-1 infection	human(A3 supertype)	[Propato (2001)]

- Long-term non-progressors (LTNPs) had strong memory resting CD8+ T-cell responses against the majority of epitopes tested (18 for the A2 supertype, 16 for the A3 supertype), while the effector cells of long-term non-progressors recognized far fewer epitopes
- Progressors had memory resting CD8+ T-cells that recognized far fewer epitopes than LTNPs
- A positive correlation between effector CD8+ T-cells and plasma viremia and a negative correlation between CD8+ effector T-cells and CD4+ T-cells was observed, which may contribute to the inability of LTNPs to clear virus

- This epitope can bind 3/5 HLA-A3 supertype alleles (A*0301, A*1101, A*3101, A*3301 and A*6801)

Nef(73–82)	Nef(73–82 BRU)	QVPLRPMTYK	HIV-1 infection	human(A3, A11, B35)	[Culmann (1991)]

- Nef CTL clones from HIV+ donors

Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK	HIV-1 infection	human(A3.1)	[Koenig (1995)]

- Alanine substitutions L76A, R77A, M79A, T80A significantly decreased immunogenicity of peptide
- Nef CTL clones (4N225) were infused into an HIV-1 infected volunteer to evaluate effects of infusion on viral load/patient health
- Infusion led to outburst of escape variants which resulted in higher viral load/accelerated disease progression

Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV-1 infection	human(A3.1)	[Betts (2000)]

- Only 4/11 HLA-A2+ HIV+ individuals had CTL that reacted to SLYNTVATL, calling into question whether it is immunodominant
- Ninety-five optimally-defined peptides from this database were used to screen for γ interferon responses to other epitopes
- 1/11 of the A2+ individuals was A3, and responded to QVPLRPMTYK as well as two other A3.1 epitopes

Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV-1 infection	human(B*0301)	[Wilson (2000)]

- Three individuals with highly focused HIV-specific CTL responses were studied during acute infection using tetramers – high frequencies of HIV-1-specific CD8+ T-cells were found prior to seroconversion, and a close temporal relationship between the number of circulating HIV-specific T-cells and viral load was also found
- All three patients were B*2705, with HLA alleles: A1, A30/31, B*2705, B35; A1, A*0301, B7, B*2705; and A*0201, A*0301, B*2705, B39
- ELISPOT was used to test a panel of CTL epitopes that had been defined earlier and was appropriate for the HLA haplotypes of the study subjects – 3/3 subjects showed a dominant response to the B*2705 epitope KRWIILGGLNK
- The subject with A*0201 had a moderately strong response to SLYNTVATL
- Weak responses were observed to A*301-RLRPGGKKK, A*301-QVPLRPMTYK, and B7-TPGPGVRYPL in the subject who was HLA A1, A*0301, B7, B*2705
- No acute response was detected to the following epitopes: A*201-ILKEPVHGV, A*301-KIRLRPGGK, A*301-AIFQSSMTK, A*301-TVYYGVPVWK, B35-EPIVGAETF, B35-HPDIVIYQY, B35-PPIPVGEIY, B35-NSSKVSQNY, B35-VPLRPMTY, B35-DPNPQEVVL

Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK		human(B27)	[Culmann(1998)]

- Optimal epitope mapped by peptide titration

Nef(73–82)	Nef(73–82 LAI)	SVPLRPMTYK	HIV-1 infection	human(B35 or C4)	[Buseyne (1993a)]

- Vertical transmission of HIV ranges from 13% to 39%
- Primary assays showed cytotoxic activity against at least one HIV protein was detected in 70% of infected children
- Epitopes recognized in five children were mapped using synthetic peptides and secondary cultures
- Patient EM13, who had a CTL response to three epitopes in Nef, was infected via blood transfusion after birth and went from CDC stage P2A to P2E during the study

Nef(74–81)	Nef(74–82)	VPLRPMTY		human(A3)	[Carreno (1992)]
• Included in HLA-A3 binding peptide competition study					
Nef(74–81)	Nef(73–82 LAI)	VPLRPMTY	HIV-1 or HIV-2 infection	human(B*3501)	[Brander & Goulder(2001)]
• C. Brander notes this is a B*3501 epitope					
Nef(74–81)	Nef(75–82)	VPLRPMTY	Peptide-HLA interaction	human(B*3501)	[Smith (1996)]
• Crystal structure of VPLRPMTY-class I B allele HLA-B*3501 complex					
Nef(74–81)	Nef( )	VPLRPMTY	HIV-1 infection	human(B*3501)	[Ostrowski (2000)]
• The role of CD4+ T-cell help in expansion of virus-specific memory CTL was studied through co-culture *ex vivo*					
• Optimal expansion of HIV-1-specific memory CTL depended on CD4+ T-cell help in 9/10 patients – CD40 ligand trimer (CD40LT) could enhance CTL in the absence of CD4+ T-cell help to a variable degree in most of patients					
• Those CTL that didn't respond to CD40LT could expand with IL-2 present, and IL-15 produced by dendritic cells also contributes					
• The T-helper epitope used for CD4+ T-cell stimulation was the universal tetanus helper epitope TET830-843 (QYIKANSKFIGITE)					
Nef(74–81)	Nef(73–82 LAI)	VPLRPMTY	HIV-1 or HIV-2 infection	human(B35)	[Culmann (1991), McMichael & Walker(1994)]
• Review of HIV CTL epitopes – defined by B35 motif found within a larger peptide					
Nef(74–81)	Nef(73–82 LAI)	VPLRPMTY	HIV-1 or HIV-2 infection	human(B35)	[Rowland-Jones (1995)]
• VPLRPMTY also recognized by CTL from HIV-2 seropositives; epitope is conserved					
Nef(74–81)	Nef( )	VPLRPMTY	HIV-1 exposed seronegative	human(B35)	[Rowland-Jones (1998a)]
• A CTL response was found in exposed but uninfected prostitutes from Nairobi using previously-defined B clade epitopes that tended to be conserved in A and D clades – such cross-reactivity could protect against both A and D and confer protection in Nairobi where both subtypes are circulating					
• The A and D subtype consensus are identical to the B clade epitope					
Nef(74–81)	Nef(75–82)	VPLRPMTY	*in vitro* stimulation	human(B35)	[Lalvani (1997)]
• A peptide-based protocol was optimized for restimulation of CTLp using optimized peptide and IL-7 concentrations – importantly this protocol does not stimulate a primary response, only secondary – peptide-specific CTLp counts could be obtained via staining with peptide-Class I tetramers					
• This peptide was one of the B35 presented test peptides used in control experiments showing that the assay gave no activity using lymphocytes from 21 healthy B35 seronegative donors					
Nef(74–81)	Nef( )	VPLRPMTY	HIV-1 exposed seronegative	human(B35)	[Rowland-Jones (1998b)]
• HIV-specific CTL were found in exposed seronegative prostitutes from Nairobi – these CTL may confer protection					
• Seroprevalence in this cohort is 90-95% and their HIV-1 exposure is among the highest in the world					
• Most isolated HIV strains are clade A in Nairobi, although clades C and D are also found – B clade epitopes are often cross-reactive, however stronger responses are frequently observed using A or D clade versions of epitopes					

- This epitope is conserved among A, B, and D clade viruses

Nef(74–81)	Nef( )	VPLRPMTY		human(B35)	[Rowland-Jones (1999)]

- CTL responses in seronegative highly HIV-exposed African female sex workers in Gambia and Nairobi were studied – these women had no δ32 deletion in CCR5
- In Gambia there is exposure to both HIV-1 and HIV-2, CTL responses to B35 epitopes in exposed, uninfected women are cross-reactive,
- HIV-2 version of this epitope is conserved: VPLRPMTY, and CTLs are cross-reactive – one of five B35 CTL epitopes that are cross-reactive, see also [Rowland-Jones (1995)]

Nef(74–81)	Nef(74–81)	VPLRPMTY	HIV-1 infection	human(B35)	[Oxenius (2000)]

- Epitope name: VPL. Patients who started therapy at acute HIV-1 infection (three with sustained therapy, two with limited therapy upon early infection) had strong HIV-specific CD4 proliferative responses and were able to maintain a CTL response even with undetectable viral load – three patients that had delayed initiation of HAART had no HIV-specific CD4 proliferative responses and lost their CTL responses when HAART was eventually given and their viral loads became undetectable
- One of two HLA B35+ among the eight study subjects recognized this epitope
- Patient SC15 (HLA A1/68, B8/35, Bw4/6, Cw4/0704) was given acute and sustained therapy and recognized epitopes PPIPVGDIY and VPLRPMTY during 331 days of HAART treatment

Nef(74–81)	Nef(75–82)	VPLRPMTY	HIV-1 exposed seronegative, HIV-1 infection	human(B35)	[Kaul (2001a)]

- ELISPOT was used to study CTL responses to a panel of 54 predefined HIV-1 epitopes in 91 HIV-1-exposed, persistently seronegative (HEPS) and 87 HIV-1-infected female Nairobi sex workers
- Responses in HEPS women tended to be lower, and focused on different epitopes with HLA presenting molecules that have previously been associated with reduced risk of infection, and there was a shift in the response in the HEPS women upon late seroconversion to epitopes recognized by the HIV-1-infected women
- 43/91 HEPS women had CD8+ responses and detection of HIV-1-specific CTL in HEPS women increased with the duration of viral exposure
- Subject ML 857 shifted from an A*6802 DTVLEDINL and B35 H/NPDIVIYQY response prior to seroconversion to a B35 PPIPVGDIY and B35 VPLRPMTY response post-seroconversion

Nef(74–82)	Nef(73–82)	VPLRPMTYK	Peptide-HLA interaction	human(A11)	[Zhang (1993)]

- Exploration of A11 binding motif

Nef(75–82)	Nef(75–82 LAI)	PLRPMTYK	HIV-1 infection	human(A*1101)	[McMichael & Walker(1994), Brander & Goulder(2001)]

- Review of HIV CTL epitopes
- C. Brander notes that this is an A*1101 epitope

Nef(77–85)	Nef(77–85 LAI)	RPMTYKAAL	HIV-1 infection	human(B*0702)	[Bauer (1997)]

- Structural constraints on the Nef protein may prevent escape
- Noted in Brander 1999, this database, to be B*0702

Nef(77–85)	Nef(77–85 LAI)	RPMTYKAAL	HIV-1 infection	human(B*0702)	[Brander & Goulder(2001)]

- C. Brander notes this is a B*0702 epitope

Nef(77–85)	Nef(75–83 IIIB)	RPMTYKAAL	HIV-1 infection	human(B7)	[Oxenius (2001b)]

- Study of tetramer staining of B7 around RPMTYKAAL gave quantitative results that were very different than functional measurements based on an ELISPOT assay
- Autologous clones were checked and 39/40 clones from two time points had the variant sequence RPMTYKGAL – tetramers based on RPMTYKGAL gave a more intense and uniform staining and bound with higher affinity to the RPMTYKGAL Vβ14 TCR

Nef(77–85)	Nef(77–85 SF2)	RPMTYKAAL	HIV-1 infection	human(B7)	[Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection
- The breadth and specificity of the response was determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response
- Number of HLA-B7+ individuals that had a CTL response to this epitope broken down by group: 1/4 group 1, 0/3 group 2, and 1/1 group 3

Nef(77–85)	Nef(77–85)	RPMTYKAAL	HIV-1 infection	human(B7)	[Day (2001)]

- Both variants RPMTYKAA[V,L] served as epitopes
- The CTL response to optimally defined CTL epitopes restricted by HLA class I A and B alleles in individuals who co-expressed HLA A2, A3, and B7 was studied in eight HIV-1-infected subjects, two with acute infection, five with chronic, and one long-term non-progressor (LTNP)
- Two to 17 epitopes were recognized in a given individual, A2-restricted CTL response tended to be narrow and never dominated the response, and 25/27 epitopes were targeted by at least one person
- Subjects with chronic HIV-1 infection recognized between 2-8 out of 11 B7-restricted epitopes
- An acute seroconvertor homozygous for the B7 allele recognized five B7-restricted epitopes
- The other acute seroconvertor failed to recognize any of the 11 B7-restricted epitopes tested
- The B7-restricted CTL response was highly variable and there was no clearly dominant epitope

Nef(79–86)	Nef(81–89 HXB3)	MTYKAALDL	Vaccine	murine(HLA-A201 transgenic)	[Sandberg (2000)]

Vaccine: *Vector/type:* DNA, peptide *Strain:* HXB3 *HIV component:* Nef *Stimulatory Agents:* Freund's adjuvant

- Ten Nef 9-mer peptides were predicted to have a strong binding affinity with HLA-A*0201 – of these, four did bind strongly by a T2 class I stabilization assay, several others bound weakly

- A CTL immune response to only 3/10 peptides was detected by a 51Cr-release assay after immunization of HLA-A201 transgenic mice with either nef DNA under the control of a CMV promotor coated on, gold particles delivered to abdominal skin by gene gun
- MTYKAALDL bound weakly to HLA-A2, but the DNA nef vaccine elicited a good CTL response

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Nef(82–91) Nef(82–91 LAI) KAAVDLSHFL HIV-1 infection human(C*0802) [Nixon (1999)]

- A patient who made a mono-specific CTL response to this Nef specific epitope was given effective anti-retroviral therapy within 90 days of infection, reducing the antigenic stimulous
- Within 7 days of therapy, his CTLp frequency dropped from 60 to 4 per million PBMC, as his viremia dropped
- The patient went from having an activated effector population (detected by CTLp and clone specific RNA) to a non-activated quiescent population (detected by the CTL-clone specific DNA)

Nef(82–91) Nef(82–91 LAI) KAAVDLSHFL HIV-1 infection human(C*0802(Cw8)) [Brander & Goulder(2001)]

- C. Brander notes this is a C*0802(Cw8) epitope

Nef(82–91) Nef(82–91 SF2) KAAVDLSHFL HIV-1 infection human(Cw8) [Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection
- The breadth and specificity of the response was determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response
- Number of HLA-Cw8+ individuals that had a CTL response to this epitope broken down by group: 1/3 group 1, 0/0 group 2, and 0/1 group 3

Nef(82–91) Nef( ) KAAVDLSHFL HIV-1 infection human(Cw8) [Altfeld (2000)]

- This epitope was mapped by ELISPOT in a study identifying new HLA-B60 epitopes, and was one of the epitopes presented by a molecule other than B60 in an HLA-B60 individual

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Nef(82–101) Nef(81–100 SF2) KAAVDLSHFLKEKGG-LEGLI HIV-1 infection human( ) [Lieberman (1997a)]

- Of 25 patients, most had CTL specific for more than one HIV-1 protein
- Eleven subjects had CTL that could recognize vaccinia-expressed LAI Nef
- Three of these 11 had CTL response to this peptide

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(82–101)	Nef( )	KAAVDLSHFLKEKGG-LEGLI	HIV-1 infection	human( )	[Altfeld (2001a)]

- HIV+ individual AC-06 was tested for reactive overlapping peptides spanning all HIV-1 proteins in an ELISPOT and was found to react with 12 peptides from 7 proteins, suggesting that the breadth of CTL responses is underestimated if accessory proteins are not included in the study
- Nef peptides PQVPLRRMTYKAAVDLSHFL, KAAVDLSHFLKEKGGLEGLI and EEEEVGFPVTPQVPLRPMTY were recognized and the first two share KAAVDLSHFL (a Cw8 epitope), the first and last share PQVPLRPMTY

Nef(83–91)	Nef(85–93 HXB3)	AALDLSHFL	Vaccine	murine(HLA-A201 transgenic)	[Sandberg (2000)]

Vaccine: *Vector/type:* DNA, peptide *Strain:* HXB3 *HIV component:* Nef *Stimulatory Agents:* Freund's adjuvant

- Ten Nef 9-mer peptides were predicted to have strong binding affinity for HLA-A*0201 – of these, four did bind strongly by a T2 class I stabilization assay, several others bound weakly
- A CTL immune response to only 3/10 peptides was detected by a 51Cr-release assay after immunization of HLA-A201 transgenic mice with nef DNA under the control of a CMV promotor, coated on gold particles delivered to abdominal skin by gene gun
- AALDLSHFL was predicted to have a strong binding capacity for HLA-A2, and did, but it was the only one of the peptides recognized that was a strong binder, the other two recognized peptides were weak binders
- AALDLSHFL was also tested by subcutaneous injection of Nef peptides in Freund's adjuvant and gave a strong response to the peptide

Nef(83–92)	Nef(81–90 93TH253 CRF01)	GAFDLSFFLK	HIV-1 infection	human(A11)	[Sriwanthana (2001)]

- Epitope name: N83-92. This was a study of HIV-1 exposed persistently seronegative (HEPS) female sex workers in Chiang Mai, northern Thailand
- HLA-A11 is very common in this population, and was enriched among the HEPS sex workers – weak CTL responses were detected in 4/7 HEPS women, and CTL responses were found in 8/8 HIV+ controls, and 0/9 HIV- women that were not exposed
- This epitope was strongly reactive in HIV+ study subjects 053 and 184 who carried HLA A11

Nef(83–92)	Nef(81–90 93TH253 CRF01)	GAFDLSFFLK	HIV-1 infection	human(A11)	[Bond (2001)]

- HLA-A11 CRF01 (called subtype E in Bond *et al.*) epitopes were identified that stimulated CTL from HIV+ female sex workers (FSW) from Northern Thailand, of whom more than half were HLA-A11 positive
- 77 possible HLA-A11 epitopes were first defined using EpiMatrix, these were screened for binding to A11 and 26 bound, and 12 of these were epitopes for CTL responses from 8 HLA-A11 positive FSWs, six were novel, six were previously identified
- This epitope was predicted by the EpiMatrix method to be likely to bind to A11, and it served as an epitope in the FSWs; it was one of the six A11 epitopes that had been previously defined
- 4/8 tested FSWs recognized this epitope
- This epitope was only conserved in CRF01 and subtype C, and exact matches were uncommon

Nef(83–94)	Nef(83–94 BRU)	AAVDLSHFLKEK	HIV-1 infection	human(A11)	[Culmann (1991)]
• Epitope defined by boundaries of overlapping peptides that stimulate Nef CTL clones					
Nef(84–91)	Nef(84–91 LAI)	AVDLSHFL	HIV-1 infection	human(Bw62)	[Culmann-Penciolelli (1994)]
Nef(84–91)	Nef(84–91)	AVDLSHFL	HIV-1 infection	human(Bw62)	[Betts (2000)]
• Only 4/11 HLA-A2+ HIV+ individuals had CTL that reacted to SLYNTVATL, calling into question whether it is immunodominant					
• Ninety-five optimally-defined peptides from this database were used to screen for γ interferon responses to other epitopes					
• 1/11 of the A2+ individuals that didn't respond to SLYNTVATL reacted with seven other epitopes including this epitope					
Nef(84–92)	Nef(84–92 LAI)	AVDLSHFLK	HIV-1 infection	human(A*1101)	[Brander & Goulder(2001)]
• C. Brander notes this is an A*1101 epitope					
Nef(84–92)	Nef(84–92 LAI)	AVDLSHFLK	HIV-1 infection	human(A11)	[McMichael & Walker(1994)]
• Review of HIV CTL epitopes					
• C. Brander notes that this is an A*1101 epitope in the 1999 database					
Nef(84–92)	Nef(84–92)	AVDLSHFLK	HIV-1 infection	human(A11)	[Betts (2000)]
• Only 4/11 HLA-A2+ HIV+ individuals had CTL that reacted to SLYNTVATL, calling into question whether it is immunodominant					
• Ninety-five optimally-defined peptides from this database were used to screen for γ interferon responses to other epitopes					
• 1/11 of the A2+ individuals that didn't respond to SLYNTVATL reacted with seven other epitopes including this epitope					
Nef(84–92)	Nef(84–92 LAI)	AVDLSHFLK	HIV-1 infection	human(A11)	[Couillin (1994), Goulder (1997a)]
• Mutational variation in HIV epitopes in individuals with appropriate HLA types can result in evasion of CTL response					
• [Goulder (1997a)] is a review of immune escape that summarizes this study					
Nef(84–92)	Nef(84–92 LAI)	AVDLSHFLK	HIV-1 infection	human(A11)	[Couillin (1995)]
• Mutations found in this epitope in HLA-A11 positive and negative donors were characterized					
Nef(84–92)	Nef(84–92)	AVDLSHFLK	HIV-1 infection	human(A11)	[Oxenius (2000)]
• Epitope name: AVD. Patients who started therapy at acute HIV-1 infection (three with sustained therapy, two with limited therapy upon early infection) had strong HIV-specific CD4 proliferative responses and were able to maintain a CTL response even with undetectable viral load – three patients that had delayed initiation of HAART had no HIV-specific CD4 proliferative responses and lost their CTL responses when HAART was eventually given and their viral loads became undetectable					
• Both of the 2/8 HLA-A11 study subjects recognized this CTL epitope					
• Patient SC19(HLA A11/12, B8/44, Cw06/0701, DR3/7, DR52/53, DQ 2/8) had a CTL response to epitopes FLKEKGGL, GEIYKRWII, ACQGVGGPGHK, AVDLSHFLK, and FNCGGEFFY that declined during therapy initiated at day 197					

	• Patient SC18(HLA A2/11, B8/44, Cw06/0701, DR3/7, DR52/53, DQ2) recognizes the epitopes ACQGVGGPGHK, QVPLRPMTYK, AVDLSHFLK, and one called QIY but not fully described – he had brief therapy upon seroconversion and has had low viral load during 600 days of follow up				
Nef(84–92)	Nef(82–90)	AVDLSHFLK	HIV-1 infection	human(A11)	[Ferrari (2000)]
	• One of the 51 HIV-1 epitopes selected by Ferrari *et al*. as good candidate CTL epitopes for vaccines by virtue of being conserved and presented by common HLA alleles				
Nef(84–92)	Nef(84–92 SF2)	AVDLSHFLK	HIV-1 infection	human(A11)	[Altfeld (2001c)]
	• Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection • The breadth and specificity of the response were determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef • Previously described and newly-defined optimal epitopes were tested for CTL response • Number of HLA-A11+ individuals that had a CTL response to this epitope broken down by group: 0/3 group 1, 0/0 group 2, and 2/2 group 3				
Nef(84–92)	Nef(84–92)	AVDLSHFLK	HIV-1 exposed seronegative, HIV-1 infection	human(A11)	[Kaul (2001a)]
	• ELISPOT was used to study CTL responses to a panel of 54 predefined HIV-1 epitopes in 91 HIV-1-exposed, persistently seronegative (HEPS) and 87 HIV-1-infected female Nairobi sex workers				
Nef(86–94)	Nef(86–94)	DLSHFLKEK	HIV-1 exposed seronegative, HIV-1 infection	human(A3)	[Kaul (2001a)]
	• ELISPOT was used to study CTL responses to a panel of 54 predefined HIV-1 epitopes in 91 HIV-1-exposed, persistently seronegative (HEPS) and 87 HIV-1-infected female Nairobi sex workers				
Nef(86–94)	Nef(84–92 LAI)	DLSHFLKEK	HIV-1 infection	human(A3.1)	[McMichael & Walker(1994)]
	• Review of HIV CTL epitopes				
Nef(86–100)	Nef(86–100 LAI)	DLSHFLKEKGGLEGL	HIV-1 infection	human(A2)	[Robertson (1993)]
	• Development of a retroviral vector (pNeoNef) to generate autologous targets				
Nef(86–100)	Nef(86–100 LAI)	DLSHFLKEKGGLEGL	HIV-1 infection	human(B35)	[Buseyne (1993b)]

Nef(86–100)	Nef(86–100 LAI)	DLSHFLKEKGGLEGL	HIV-1 infection	human(B35 or C4)	[Buseyne (1993a)]

- Vertical transmission of HIV ranges from 13% to 39%
- Primary assays showed cytotoxic activity against at least one HIV protein was detected in 70% of infected children
- Epitopes recognized in five children were mapped using synthetic peptides and secondary cultures
- Patient EM13, who had a CTL response to three epitopes in Nef, was infected via blood transfusion after birth and went from CDC stage P2A to P2E during the study

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Nef(87–102)	Nef( )	FSHFLKEKGGLEGLIY		human( )	[Jubier-Maurin (1999)]

- 41 new HIV-1 strains describing envelope subtypes of HIV-1 A-H were genetically characterized in the nef region – 34 subtypes were classified in the same subtype in nef and env and 7 of the 41 strains were recombinants
- This region was defined as a CTL epitope region that is conserved among HIV-1 M group subtypes

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Nef(90–97)	Nef(92–99)	FLKEKGGL	HIV-1 infection	human(B8)	[Oxenius (2000)]

- Epitope name: FLK. Patients who started therapy at acute HIV-1 infection (three with sustained therapy, two with limited therapy upon early infection) had strong HIV-specific CD4 proliferative responses and were able to maintain a CTL response even with undetectable viral load – three patients that had delayed initiation of HAART had no HIV-specific CD4 proliferative responses and lost their CTL responses when HAART was eventually given and their viral loads became undetectable
- Six of the 7/8 study subjects that were HLA B8 recognized this early dominant CTL epitope
- Patient SC2 (HLA A1, B7/8, Cw0701/0702, DR4/53, DQ7) had CTL responses against epitopes FLKEKGGL, GPKVKQWPL, and GEIYKRWII – FLKEKGGL tetramer staining steadily declined and at day 1340 the FLKEKGGL stained cells were no longer detected and the escape mutant FLKENGGI was found in 8/10 clones
- Patient SC9 (HLA A1/2, B8/13, Cw0/0701, DR2/11, DQ6/7) had a CTL response against epitopes FLKEKGGL, ILKEPVHGV, SQRRQDILDLWIYHTQGYFPDWQNY, and GEIYKRWII and all responses declined during therapy initiated at day 390 but were restored when therapy became intermittent
- Patient SC19(HLA A11/12, B8/44, Cw06/0701, DR3/7, DR52/53, DQ 2/8) had a CTL response to epitopes FLKEKGGL, GEIYKRWII, ACQGVGGPGHK, AVDLSHFLK, and FNCGGEFFY that declined during therapy initiated at day 197
- Patient SC10(HLA A1/3, B8/35, DR1/8, DQ 4/5) had sustained therapy started during acute infection and maintained an immunodominant response to FLKEKGGL and a response to GEIYKRWII through day 1088
- Patient SC12(HLA A1, B8/39, Cw0701/0702, DR2/3, DR51/52, DQ2/6) had sustained therapy started during acute infection and maintained an immunodominant response to FLKEKGGL throughout and minor responses to GEIYKRWII, DCKTILKAL, GGKKKYKLK – GEIYKRWII and GGKKKYKLK responses were stimulated by a brief period off therapy
- Patient SC11(HLA A1, B8, Cw0201, DR3/11, DR52, DQ2/7) started therapy early, remained on therapy for 40 days, then reinitiated HAART at day 640, and had a CTL response to FLKEKGGL, GPKVKQWPL, and GEIYKRWII throughout and received a benefit from the early limited course therapy

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Nef(90–97)	Nef(89–97)	FLKEKGGL	HIV-1 infection	human( )	[Betts (2000)]

- Only 4/11 HLA-A2+ HIV+ individuals had CTL that reacted to SLYNTVATL, calling into question whether it is immunodominant
- Ninety-five optimally-defined peptides from this database were used to screen for γ interferon responses to other epitopes

- 1/11 of the A2+ individuals that responded to SLYNTVATL reacted with seven other epitopes including this epitope previously described as presented by B8

Nef(90–97) Nef( ) FLKEKGGL HIV-1 infection human(A3) [Ostrowski (2000)]

- The role of CD4+ T-cell help in expansion of virus-specific memory CTL was studied through co-culture *ex vivo*
- Optimal expansion of HIV-1-specific memory CTL depended on CD4+ T-cell help in 9/10 patients – CD40 ligand trimer (CD40LT) could enhance CTL in the absence of CD4+ T-cell help to a variable degree in most of patients
- Those CTL that didn't respond to CD40LT could expand with IL-2 present, and IL-15 produced by dendritic cells also contributes
- The T-helper epitope used for CD4+ T-cell stimulation was the universal tetanus helper epitope TET830-843 (QYIKANSKFIGITE)

Nef(90–97) Nef(89–97 LAI) FLKEKGGL HIV-1 infection human(B*0801) [Brander & Goulder(2001)]

- C. Brander notes this is a B*0801 epitope

Nef(90–97) Nef(89–97 LAI) FLKEKGGL HIV-1 infection human(B8) [Price (1997)]

- CTL escape variants appeared over time in HLA-B8 HIV-1+ individual, providing evidence of immune escape
- Most variants appear at position 5, an anchor residue
- FLKE(E,N or Q)GGL showed reduced binding efficiency and recognition
- Double mutants (FIKENGGL, FLEENGGL, and FLKGNGGL) completely escaped recognition
- [Goulder (1997a)] is a review of immune escape that summarizes this study in the context of CTL escape to fixation

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Nef(90–97) Nef(90–97 IIIB) FLKEKGGL HIV-1 infection human(B8) [Spiegel (1999)]

- Study examines the effect of highly active antiretroviral therapy (HAART) on HIV-1 plasma viral load, CTLp and CTLe frequencies in 8 infected children
- CTLp (precursors) were measured by stimulating in culture and assaying using 51Cr release, against vaccina expressed IIIB Env, Gag, Pol, Nef
- B7-FLKEKGGL tetramer complex was used for one of the children that was HLA-B7, and this infant showed a vigorous response (> 4% of CD8+ T-cells) at 9 months of age
- HIV-1 specific CTL responses initially increased in children with complete viral suppression, but then decreased, suggesting viral replication is needed to maintain CTL responses

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Nef(90–97) Nef( ) FLKEKGGL Vaccine human(B8) [Hanke (1998a), Hanke (1998b)]

Vaccine: *Vector/type:* vaccinia *HIV component:* polyepitope

- This epitope was shown to be processed and presented to appropriate CTL clones upon infection of human target cells with vaccinia virus Ankara (VVA) carrying 20 HIV-1 epitopes recognized by humans

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Nef(90–97) Nef(88–95) FLKEKGGL HIV-1 infection human(B8) [Goulder (1997g)]

- Natural variants for this epitope have been observed in several donors
- Substitutions Q5, N5, E5 that alter anchor position 5 are not well recognized
- Substitution I2 binds well to B8 and is recognized

Nef(90–97)	Nef(90–97)	FLKEKGGL	HIV-1 infection	human(B8)	[Dyer (1999)]

- CTL specific responses were measured over a 1.3 to 1.5 year period in members of the Sydney Blood Bank Cohort (SBBC) who had been infected with a natural attenuated strain of HIV-1 which was Nef-defective
- Some of these patients had prolonged high levels of CTL effector and memory cells despite low viral load

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Nef(90–97)	Nef( )	FLKEKGGL	HIV-1 infection	human(B8)	[Goulder (2001b)]

- Epitope name: FL8. This peptide elicited a weak CTL response during acute HIV-1 infection in patient PI004
- Three CTL responses, to epitopes TSTLQEQIGW, ISPRTLNAW, and KAFSPEVIPMF, were evident early after infection; CTL responses to SLYNTVATL, QASQEVKNW, EIYKRWII, and FLKEKGGL were detectable at 5 months post-infection and beyond
- FL8 was recognized in an additional patient, AC29, in chronic infection

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Nef(90–97)	Nef(92–99)	FLKEKGGL	HIV-1 infection	human(B8)	[Oxenius (2001a)]

- Epitope name: FLK. Characterization of specific CTL phenotype patterns in response to variation of the virus load in response to antiviral therapy in 3 patients with chronic HIV-1 infection
- CTL activation in response to increasing viral load sequential, and co-segregated with apoptosis only during later stages of the response, suggesting antigen-specific cell-death is restricted to distinct CTL sub-populations

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Nef(90–97)	Nef( )	FLKEKGGL	HIV-1 infection	human(B8)	[Kostense (2001)]

- HLA tetramers to six epitopes were used to study HLA-A2, B8 and B57 CTL in 54 patients – HIV-specific tetramer positive cells were inversely correlated with viral load in patients with high CD4, but in patients with CD4 T-cells below 400 high tetramer frequencies were found despite high viral load
- Most patients have high levels of HIV-specific T-cell expansions, but many of these cells aren't functional
- In 15 of the patients, the proportion of IFNγ producing tetramer cells correlated with AIDS-free survival
- Stimulation with HLA-B8 p24 and Nef epitopes significantly increased Nef-specific T-cell numbers in 2 patients (748 and 1113)
- There were more functional IFN-γ producing Nef-specific T-cells within the T-cell population than there were active p24 Gag-specific T-cells
- No correlation between elevated numbers of Nef-specific CTL cells and plasma viral load was observed

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Nef(90–97)	Nef(88–95)	FLKEKGGL	HIV-1 infection	human(B8)	[Ferrari (2000)]

- One of the 51 HIV-1 epitopes selected by Ferrari *et al*. as good candidate CTL epitopes for vaccines by virtue of being conserved and presented by common HLA alleles

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Nef(90–97)	Nef(88–95 SF2)	FLKEKGGL	HIV-1 infection	human(B8)	[Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection
- The breadth and specificity of the response were determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response

- Number of HLA-B8+ individuals that had a CTL response to this epitope broken down by group: 3/3 group 1, 1/3 group 2, and 1/2 group 3

Nef(90–97) Nef(89–97) FLKEKGGL HIV-1 infection human(B8) [Appay (2000)]

- Combined tetramer and intracellular cytokine staining was used to study the function of circulating CD8+ T-cells specific for HIV and CMV
- HIV-specific CD8+ T-cells expressed lower levels of perforin than CMV-specific CD8+ T-cells from the same donor, and this was associated with persistent CD27 expression on HIV-specific cells, suggesting impaired maturation
- In most donors, between 50% and 95% of the activated virus-specific CD8+ T-cells produced IFN-γ and MIP-1β with a distinct subset that failed to produce TNF-α

Nef(90–97) Nef(90–97) FLKEKGGL HIV-1 infection human(B8) [Day (2001)]

- B8-restricted CTL accounted for about 1/3 of the total CTL response in one individual
- The response to FLKEKGGL was the second highest response in magnitude compared to all the HLA class I A- and B-restricted epitopes tested in this individual

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Nef(90–97) Nef( ) FLKEKGGL HIV-1 infection human(B8) [Goulder (2000b)]

- Tetramer assays were compared with three functional assays in 42 people with chronic HIV infection: ELISPOT, intracellular cytokine staining, and precursor frequency (limiting dilution assay [LDA])
- HIV-specific tetramer staining CTLs appeared to be active, and inert CTL were not found to play a significant role in chronic pediatric or adult HIV infection

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Nef(92–100) ( ) KEKGGLEGL human(B*4001) [Brander & Goulder(2001)]

- C. Brander notes this is a B*4001,B60 epitope

Nef(92–100) Nef(91–99 BRU) KEKGGLEGL HIV-1 infection human(B*4002) [Mulligan (2001)]

- Epitope N10 from Patient 07118 with HLA genotypes A*0209, A*3201, B*4002, B*5301, Cw*0202, Cw*0401
- Epitope N10 Patient 07118 has 4 more optimal peptides P55, PIQKETWETW with HLA A*3201; G21 and G22, AEWDRVHPV with HLA B*4002; G31, QASQEVKNW with HLA B*5301;G43, TERQANFL with HLA B*4002

Nef(92–100) Nef(90–98 SF2) KEKGGLEGL HIV-1 infection human(B60) [Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection
- The breadth and specificity of the response were determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response
- Number of HLA-B60+ individuals that had a CTL response to this epitope broken down by group: 2/2 group 1, 1/1 group 2, and 0/0 group 3

Nef(92–100)	Nef( )	KEKGGLEGL	HIV-1 infection	human(B60(B*4001)	[Altfeld (2000)]

- This epitope was the dominant B60 (encoded by B*4001) response in 6/8 HLA-B60 individuals, and recognized in all eight
- This epitope was also recognized two expressing HLA-B61 individuals (B61 is usually encoded by B*4002, but this study did not distinguish between B*4002, B*4003, B*4004, B*4006, and B*4008)
- ELISPOT was a rapid an effective method that was used to define five novel B60 epitopes
- HLA-B60 is present in 10-20% of the Caucasoid population and B60/B61 are very common in Asian populations

Nef(92–100)	Nef(92–100)	KEKGGLEGL	HIV-1 infection	human(B60/B61)	[Day (2001)]

- No immunodominant responses were detected to five B61-restricted epitopes tested in an HLA-B61 subject
- All five B60-restricted epitopes were reactive in an HLA-B60+ subject, and the B60-restricted responses together contributed over one-third of the total CTL response

Nef(92–112)	Nef( )	KEKGGLEGLIHSQRRQ-DILDL	HIV-1 infection	human( )	[Altfeld (2000)]

- This epitope was mapped by ELISPOT in a study identifying new HLA-B60 epitopes, and was one of the epitopes presented by a molecule other than B60 in an HLA-B60 individual
- The response to the peptide was CD8 dependent, but the HLA presenting molecule and optimal epitope were not determined

Nef(92–112)	Nef( )	KEKGGLEGLIHSQRRQ-DILDL	HIV-1 infection	human( )	[Altfeld (2000)]

- This epitope was mapped by ELISPOT in a study identifying new HLA-B60 epitopes, and was one of the epitopes presented by a molecule other than B60 in an HLA-B60 individual
- The response to the peptide was CD8 dependent, but the HLA presenting molecule and optimal epitope were not determined

Nef(93–106)	Nef(93–106 BRU)	EKGGLEGLIHSQRR	HIV-1 infection	human(A1, B8)	[Hadida (1992)]

- HIV-1 specific CTLs detected in lymphoid organs of HIV-1 infected patients

Nef(102–115)	Nef(102–115 LAI)	HSQRRQDILDLWIY	HIV-1 infection	human(B7)	[Goulder (1997e), Goulder (1997a)]

- Identical twin hemophiliac brothers were both infected with the same batch of factor VIII
- One had a strong response to this peptide, the other did not
- [Goulder (1997a)] is a review of immune escape that summarizes this study

Nef(102–121)	Nef(101–120 SF2)	HSQRRQDILDLQIYHT-QGYF	HIV-1 infection	human( )	[Lieberman (1997a)]

- Of 25 patients, most had CTL specific for more than one HIV-1 protein
- Eleven subjects had CTL that could recognize vaccinia-expressed LAI Nef
- Two of these 11 had CTL response to this peptide
- The responding subjects were HLA-A2, A3, B8, B62 and HLA-A2, B21

Nef(103–127)	Nef(103–127 PV22)	SQRRQDILDLWIYHTQ-GYFPDWQNY	HIV-1 infection	human(B13)	[Jassoy (1993)]
• HIV-1 specific CTLs release γ-IFN, and α- and β-TNF					
Nef(103–127)	Nef(103–127)	SQRRQDILDLWIYHTQ-GYFPDWQNY	HIV-1 infection	human(B13)	[Oxenius (2000)]
• Epitope name: SQR. Patients who started therapy at acute HIV-1 infection (three with sustained therapy, two with limited therapy upon early infection) had strong HIV-specific CD4 proliferative responses and were able to maintain a CTL response even with undetectable viral load – three patients that had delayed initiation of HAART had no HIV-specific CD4 proliferative responses and lost their CTL responses when HAART was eventually given and their viral loads became undetectable • The only study subject out of eight that was HLA B13+ recognized this epitope • Patient SC9 (HLA A1/2, B8/13, Cw0/0701, DR2/11, DQ6/7) had a CTL response against epitopes FLKEKGGL, ILKEPVHGV, SQRRQDILDLWIYHTQGYFPDWQNY, and GEIYKRWII and all responses declined during therapy initiated at day 390 but were restored when therapy became intermittent				
Nef(105–114)	Nef(105–114 LAI)	RRQDILDLWI	HIV-1 infection	human(B*2705)	[Goulder (1997c)]
• Defined as optimal epitope from within reactive peptide HSQRRQDILDLWIYHTQGYF [Nef(102-121 LAI)] • HLA-B*2705 is associated with slow HIV disease progression • The HLA-B*2705 binding motif includes R at position 2, and L in the C-term position					
Nef(105–114)	Nef(105–114 LAI)	RRQDILDLWI	HIV-1 infection	human(B*2705)	[Brander & Goulder(2001)]
• C. Brander notes this is a B*2705 epitope					
Nef(105–114)	Nef(105–114 SF2)	RRQDILDLWI	HIV-1 infection	human(B27)	[Altfeld (2001c)]
• Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection • The breadth and specificity of the response were determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef • Previously described and newly-defined optimal epitopes were tested for CTL response • Number of HLA-B27+ individuals that had a CTL response to this epitope broken down by group: 0/0 group 1, 0/0 group 2, and 1/1 group 3					
Nef(105–114)	Nef(105–114)	RRQDILDLWI	HIV-1 infection	human(B27)	[Day (2001)]
• B27-restricted CTL response was strongest to this epitope in one individual					
Nef(106–115)	( )	RQDILDLWIY		(B7)	[Brander & Goulder(2001), Goulder(1999)]

Nef(108–115)	Nef(107–114 BRU)	DILDLWIF	HIV-1 infection	human(Cw*0701, Cw*0706)	[Mulligan (2001)]
	• Epitope N11 from Patient 02112 with HLA genotypes A*3303, A*2601, B*5801, B*8201, Cw*0302, Cw*07(01, 06)				
	• Epitope N11 Patient 02112 has an other optimal peptide P61, ETKLGKAGY with HLA A*2601				
Nef(112–133)	Nef(111–132)	LWIYHTQGYFPDWQN-YTPGPGV	*in vitro* stimulation	human( )	[Lieberman (1995)]
	• HIV-specific CTL lines developed by *ex vivo* stimulation with peptide				
Nef(112–133)	Nef(111–132 SF2)	LWIYHTQGYFPDWQN-YTPGPGV	HIV-1 infection	human( )	[Lieberman (1997a)]
	• Of 25 patients, most had CTL specific for more than one HIV-1 protein				
	• Eleven subjects had CTL that could recognize vaccinia-expressed LAI Nef				
	• Four of these 11 had CTL response to this peptide				
	• The responding subjects were HLA-A2, B21; HLA-A1, A3, B7, B15; HLA-A2, A26, B7, B38				
Nef(112–133)	Nef(111–132 SF2)	LWIYHTQGYFPDWQN-YTPGPGV	HIV-1 infection	human( )	[Lieberman (1997b)]
	• CTL expanded *ex vivo* were later infused into HIV-1 infected patients				
Nef(113–125)	Nef(113–125 BRU)	WIYHTQGYFPDWQ	HIV-1 infection	human(B17)	[Culmann (1989)]
	• Nef CTL clones from HIV+ donors				
Nef(113–126)	Nef( )	VYHTQGYFPDWQNY	HIV-1 infection	human( )	[Jubier-Maurin (1999)]
Nef(113–128)	Nef(113–128 BRU)	WIYHTQGYFPDWQNY-T	HIV-1 infection	human(A1)	[Hadida (1992)]
	• HIV-1 specific CTLs detected in lymphoid organs of HIV-1 infected patients				
Nef(113–128)	Nef(113–128 LAI)	WIYHTQGYFPDWQNY-T	HIV-1 infection	human(A1)	[Mollet (2000)]
	• Epitope name: N2. A panel of 16 epitopes covering 15 class I alleles was tested in 14 HIV+ patients from an unselected Caucasian population treated with HAART, using CD8+ cell IFNγ production to measure responses				
	• In general, during the first month of treatment viral load decreased and frequencies of HIV-specific CTL tripled and broadened – eight new HIV specificities that were not previously detectable were newly detected, as were CMV specific CD8+ PBL – but with continued viral suppression, HIV-specific responses diminished				
	• Viral rebounds gave different patterns of response: increases or decreases in pre-existing response, new specificities, or no change				
Nef(115–125)	Nef(115–125 BRU)	YHTQGYFPDWQ	HIV-1 infection	human(B17)	[Culmann (1991)]
	• Nef CTL clones from HIV+ donors				

Nef(116–125)	Nef(116–125 BRU)	HTQGYFPDWQ	HIV-1 infection	human(B*5701)	[Brander & Goulder(2001)]
• C. Brander notes this is a B*5701 epitope					
Nef(116–125)	Nef(116–125)	HTQGYFPDWQ	HIV-1 infection	human(B57)	[Betts (2000)]
• Only 4/11 HLA-A2+ HIV+ individuals had CTL that reacted to SLYNTVATL, calling into question whether it is immunodominant • Ninety-five optimally-defined peptides from this database were used to screen for γ interferon responses to other epitopes • One of the individuals that was HLA-A2+, but otherwise of unknown HLA type, and reacted with seven epitopes including this one					
Nef(116–125)	Nef(116–125 BRU)	HTQGYFPDWQ	HIV-1 infection	human(B57)	[Culmann (1991)]
• Nef CTL clones from HIV+ donors, optimal peptide mapped					
Nef(116–125)	Nef(116–125)	HTQGYFPDWQ	HIV-1 infection	human(B57)	[Oxenius (2000)]
• Epitope name: HTQ. Patients who started therapy at acute HIV-1 infection (three with sustained therapy, two with limited therapy upon early infection) had strong HIV-specific CD4 proliferative responses and were able to maintain a CTL response even with undetectable viral load – three patients that had delayed initiation of HAART had no HIV-specific CD4 proliferative responses and lost their CTL responses when HAART was eventually given and their viral loads became undetectable • None of the 8 study subjects recognized this epitope but none were HLA B57+					
Nef(117–127)	Nef(117–127)	TQGYFPDWQNY	HIV-1 infection	human( )	[Betts (2000)]
• Only 4/11 HLA-A2+ HIV+ individuals had CTL that reacted to SLYNTVATL, calling into question whether it is immunodominant • Ninety-five optimally-defined peptides from this database were used to screen for γ interferon responses to other epitopes • 1/11 of the A2+ individuals was HLA A*0205/A*0208, A30, B27, B44 but responded to HLA Bw62 epitope TQGYFPDWQNY, and one of the other individuals that was A2+, but otherwise of unknown HLA type, reacted with seven epitopes including this one					
Nef(117–127)	Nef(117–127 LAI)	TQGYFPDWQNY	HIV-1 infection	human(B*1501)	[Brander & Goulder(2001)]
• C. Brander notes this is a B*1501 epitope					
Nef(117–127)	Nef(117–127)	TQGYFPDWQNY	HIV-1 infection	human(B62)	[Day (2001)]
• No immunodominant responses were detected to four B62-restricted epitopes tested					
Nef(117–127)	Nef(117–127 LAI)	TQGYFPDWQNY	HIV-1 infection	human(Bw62)	[Culmann(1998)]
• Optimal peptide defined by titration					
Nef(117–128)	Nef(117–128 BRU)	TQGYFPDWQNYT	HIV-1 infection	human(B17, B37)	[Culmann (1991)]
• Nef CTL clones from HIV+ donors					
Nef(117–147)	Nef(117–147 LAI)	TQGYFPDWQNYTPGP-GVRYPLTFGWCYKLVP	Vaccine	human( )	[Gahery-Segard (2000)]

Vaccine: *Vector/type:* lipopeptide *HIV component:* six peptides

- Anti-HIV lipopeptide vaccine consisting of six long amino acid peptides derived from Nef, Gag and Env HIV-1 proteins modified by a palmitoyl chain was administered in a phase I trial
- A CD4+ T-cell proliferative response to at least one of the six peptides was observed in 9/10 vaccinees – 1/10 reacted to this Nef peptide
- 9/12 tested mounted a CTL response to at least one of the six peptides; each of the six peptides elicited a CTL response in at least one individual
- 10/12 tested had an IgG response to this peptide

Nef(118–127)	Nef(118–127 LAI)	QGYFPDWQNY		human(Bw62)	[McMichael & Walker(1994)]

- Review of HIV CTL epitopes

Nef(120–128)	Nef(120–128)	YFPDWQNYT	HIV-1 infection	human( )	[Betts (2000)]

- Only 4/11 HLA-A2+ HIV+ individuals had CTL that reacted to SLYNTVATL, calling into question whether it is immunodominant
- Ninety-five optimally-defined peptides from this database were used to screen for γ interferon responses to other epitopes
- 1/11 of the A2+ individuals was HLA A*0205/A*0208, A30, B27, B44 but responded to HLA B37 epitope IYKRWIILGL, and one of the other individuals that was A2+, but otherwise of unknown HLA type, reacted with seven epitopes including this one

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Nef(120–128)	Nef(118–126 SF2)	YFPDWQNYT	HIV-1 infection	human(A1)	[Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection
- The breadth and specificity of the response were determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response
- Number of HLA-A1+ individuals that had a CTL response to this epitope broken down by group: 0/3 group 1, 0/2 group 2, and 1/2 group 3

Nef(120–128)	Nef(120–128 LAI)	YFPDWQNYT	HIV-1 infection	human(B*3701)	[Brander & Goulder(2001)]

- C. Brander notes this is a B*3701 and B*5701 epitope

Nef(120–128)	Nef(120–128 LAI)	YFPDWQNYT	HIV-1 infection	human(B*5701)	[Brander & Goulder(2001)]

- C. Brander notes this is a B*5701 epitope
- Subtype of B57 not determined

Nef(120–128)	Nef(120–128 IIIB)	FFPDWKNYT	HIV-1 infection	human(B15)	[Wilson (1999a)]

- This study describes maternal CTL responses in the context of mother-to-infant transmission
- Detection of CTL escape mutants in the mother was associated with transmission, but the CTL-susceptible forms of the virus tended to be found in infected infants
- LFPDWKNYT is an escape mutant

Nef(120–128)	Nef(120–128 LAI)	YFPDWQNYT	HIV-1 infection	human(B37,B57)	[Culmann(1998)]
• Nef CTL clones from HIV+ donors – optimum peptide mapped by titration					
Nef(120–144)	Nef(120–144 SF2)	YFPDWQNYTPGPGIR-YPLTFGWCYK	HIV-1 infection	human(A24)	[Jassoy (1992)]
• Epitope recognized by CTL clone derived from CSF					
Nef(122–141)	Nef(121–140 SF2)	PDWQNYTPGPGVRYP-LTFGW	HIV-1 infection	human( )	[Lieberman (1997a)]
• Of 25 patients, most had CTL specific for more than one HIV-1 protein • Eleven subjects had CTL that could recognize vaccinia-expressed LAI Nef • Three of these 11 had CTL response to this peptide • The responding subjects were HLA-A2, B21; HLA-A3, A24, B7, B38					
Nef(123–137)	Nef(123–137 IIIB)	QWQNYTPGPGVRYPL	HIV-1 infection	human( )	[Wilson (1996)]
• Epitope defined in the context of the Pediatric AIDS Foundation ARIEL Project, a mother-infant HIV transmission study • FFPDYTPGPGTRFPL and FFPDYKPGPGTRFPL, naturally occurring variants, were found in the mother and are not recognized • LFPDYKPGPGTRFPL and FFPDYKPGPGTRFPL, naturally occurring variants, were found in the infant and are not recognized					
Nef(126–138)	Nef(126–138 BRU)	NYTPGPGVRYPLT	HIV-1 infection	human(B7)	[Culmann (1991)]
• Nef CTL clones from HIV+ donors					
Nef(128–135)	Nef(128–135 LAI)	TPGPGVRY	*in vitro* stimulation	human(B*0702)	[Lucchiari-Hartz (2000)]
• Five naturally processed MHC class I ligands were identified in Nef in the conserved immunogenic region Nef between 123-152 • All five could be transported by TAP, and 4/5 had N-termini that were major cleavage points for the proteasome, only one had extended precursor fragments • Both TPGPGVRYPL and TPGPGVRY are naturally processed ligands that can be eluted from HLA-B7 molecules, both are recognized by the same CTL, and both peptides seem to be the direct product of a proteasomal digest • The peptide TPGPGVRY is present in a high copy number, TPGPGVRYPL at a more moderate level, possibly due to a major cleavage site between the Y and P					
Nef(128–137)	Nef( )	TPGPGIRYPL	HIV-1 infection	human( )	[Kaul (2001b)]
• This study examines CTL responses in HIV-exposed, persistently seronegative individuals, HEPS, who eventually seroconverted – 11/114 HEPS Nairobi sex workers eventually seroconverted, and for six of these HIV CTL reactive epitopes had been defined while seronegative • The epidemiological factor associated with seroconversion was stopping sex work and HIV-specific CTL activity declines when HEPS sex workers stop working for a period or retire • This epitope was recognized by 1/22 HEPS control sex workers, ML851					

Nef(128–137)	Nef(128–137 LAI)	TPGPGVRYPL	HIV-1 infection	human(B*0702)	[Brander & Goulder(2001)]

- C. Brander notes this is a B*0702 epitope

Nef(128–137)	Nef(128–137 LAI)	TPGPGVRYPL	*in vitro* stimulation	human(B*0702)	[Lucchiari-Hartz (2000)]

- Five naturally processed MHC class I ligands were identified in Nef in the conserved immunogenic region Nef between 123-152
- All five could be transported by TAP, and 4/5 had N-termini that were major cleavage points for the proteasome, only one had extended precursor fragments
- Both TPGPGVRYPL and TPGPGVRY are naturally processed ligands that can be eluted from HLA-B7 molecules, both are recognized by the same CTL, and both peptides seem to be the direct product of a proteasomal digest
- The peptide TPGPGVRY is present in a high copy number, TPGPGVRYPL at a more moderate level, possibly due to a major cleavage site between the Y and P

Nef(128–137)	Nef(128–137 LAI)	TPGPGVRYPL		human(B*4201)	[Brander & Goulder(2001)]

- C. Brander notes this is a B*4201 epitope

Nef(128–137)	( )	TPGPGVRYPL	HIV-1 infection	human(B7)	[Wilson (2000)]

- Three individuals with highly focused HIV-specific CTL responses were studied during acute infection using tetramers – high frequencies of HIV-1-specific CD8+ T-cells were found prior to seroconversion, and a close temporal relationship between the number of circulating HIV-specific T-cells and viral load was also found
- All three patients were B*2705, with HLA alleles: A1, A30/31, B*2705, B35; A1, A*0301, B7, B*2705; and A*0201, A*0301, B*2705, B39
- ELISPOT was used to test a panel of CTL epitopes that had been defined earlier and was appropriate for the HLA haplotypes of the study subjects – 3/3 subjects showed a dominant response to the B*2705 epitope KRWIILGGLNK
- The subject with A*0201 had a moderately strong response to SLYNTVATL
- Weak responses were observed to A*301-RLRPGGKKK, A*301-QVPLRPMTYK, and B7-TPGPGVRYPL in the subject who was HLA A1, A*0301, B7, B*2705
- No acute response was detected to the following epitopes: A*201-ILKEPVHGV, A*301-KIRLRPGGK, A*301-AIFQSSMTK, A*301-TVYYGVPVWK, B35-EPIVGAETF, B35-HPDIVIYQY, B35-PPIPVGEIY, B35-NSSKVSQNY, B35-VPLRPMTY, B35-DPNPQEVVL

Nef(128–137)	Nef(128–137 LAI)	TPGPGVRYPL	HIV-1 infection	human(B7)	[Haas (1996), Haas (1997)]

- There was a high degree of variation in three CTL epitopes in Nef in four slow and non-progressors, and variant specific CTLs arose over time to eliminate variants, indicating immune selection
- The epitope position was taken from [Haas (1997)]

Nef(128–137)	Nef( )	TPGPGVRYPL	HIV-1-exposed seronegative	human(B7)	[Rowland-Jones (1998a)]

- A CTL response was found in exposed but uninfected prostitutes from Nairobi using previously-defined B clade epitopes that tended to be conserved in A and D clades – such cross-reactivity could protect against both A and D and confer protection in Nairobi where both subtypes are circulating

- The D subtype consensus is identical to the B clade epitope
- The A subtype consensus is TPGPGIRYPL

Nef(128–137)	Nef( )	TPGPGVRYPL	HIV-1-exposed seronegative	human(B7)	[Rowland-Jones (1998b)]

- HIV-specific CTL were found in exposed seronegative prostitutes from Nairobi – these CTL may confer protection
- Seroprevalence in this cohort is 90-95% and their HIV-1 exposure is among the highest in the world
- Most isolated HIV strains are clade A in Nairobi, although clades C and D are also found – B clade epitopes are often cross-reactive, however stronger responses are frequently observed using A or D clade versions of epitopes
- This epitope is conserved among B and D clade viruses
- The clade A version of the epitope: TPGPGIRYPL

Nef(128–137)	Nef(128–137)	TPGPGVRYPL	*in vitro* stimulation	human(B7)	[Wilson (1999b)]

- Dendritic cells are the most potent for priming T-cell responses – DCs can stimulate autologous CTL responses from T-cells cultured from HIV negative donors
- Th1-biasing cytokines IL-12 or IFNα enhance CTL responses *in vitro* whether the epitope is delivered by pulsing from peptide, or expressed from within
- CTL from a B7 donor displayed no reactivity to this epitope, although it had been immunodominant in another study [Haas (1996)]

Nef(128–137)	Nef(128–137 SF2)	TPGPGVRYPL	HIV-1 infection	human(B7)	[Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection
- The breadth and specificity of the response were determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response
- Number of HLA-B7+ individuals that had a CTL response to this epitope broken down by group: 0/4 group 1, 0/3 group 2, and 1/1 group 3

Nef(128–137)	Nef(128–137)	TPGPGVRYPL	HIV-1-exposed seronegative, HIV-1 infection	human(B7)	[Kaul (2001a)]

- ELISPOT was used to study CTL responses to a panel of 54 predefined HIV-1 epitopes in 91 HIV-1-exposed, persistently seronegative (HEPS) and 87 HIV-1-infected female Nairobi sex workers
- Responses in HEPS women tended to be lower, and focused on different epitopes with HLA presenting molecules that have previously been associated with reduced risk of infection, and there was a shift in the response in the HEPS women upon late seroconversion to epitopes recognized by the HIV-1-infected women
- 43/91 HEPS women had CD8+ responses and detection of HIV-1-specific CTL in HEPS women increased with the duration of viral exposure

- Among HLA-B7 women, 4/5 HEPS and 5/6 HIV-1-infected women recognized this epitope
- The dominant response to this HLA allele was to this epitope in 3 of the 4/5 HEPS cases and in 2 of the 5/6 HIV-1-infected women
- Subject ML 1203 started with CTL responses to A*6802 DTVLEDINL and to B7 FPVTPQVPLR prior to seroconversion, and upon seroconversion acquired additional responses to A*6802 ETAYFILKL which became dominant, B7 TPGPGV/IRYPL, B7 IPRRIRQGL, and B7 SPRTLNAWV

Nef(128–137) Nef(128–137) TPGPGVRYPL HIV-1 infection human(B7) [Appay (2000)]

- Combined tetramer and intracellular cytokine staining was used to study the function of circulating CD8+ T-cells specific for HIV and CMV
- HIV-specific CD8+ T-cells expressed lower levels of perforin than CMV-specific CD8+ T-cells from the same donor, and this was associated with persistent CD27 expression on HIV-specific cells, suggesting impaired maturation
- In most donors, between 50% and 95% of the activated virus-specific CD8+ T-cells produced IFN-γ and MIP-1β with a distinct subset that failed to produce TNF-α

Nef(128–137) Nef(128–137) TPGPGVRYPL HIV-1 infection human(B7) [Day (2001)]

- The CTL response to optimally defined CTL epitopes restricted by HLA class I A and B alleles in individuals who co-expressed HLA A2, A3, and B7 was studied in eight HIV-1-infected subjects, two with acute infection, five with chronic, and one long-term non-progressor (LTNP)
- Two to 17 epitopes were recognized in a given individual, A2-restricted CTL response tended to be narrow and never dominated the response, and 25/27 epitopes were targeted by at least one person
- Subjects with chronic HIV-1 infection recognized between 2-8 out of 11 B7-restricted epitopes
- An acute seroconvertor homozygous for the B7 allele recognized five B7-restricted epitopes
- The other acute seroconvertor failed to recognize any of the 11 B7-restricted epitopes tested
- The B7-restricted CTL response was highly variable and there was no clearly dominant epitope

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Nef(128–137) Nef( ) TPGPGVRYPL HIV-1-exposed seronegative human(B7(*8101)) [Rowland-Jones (1998b)]

- HIV-specific CTL were found in exposed seronegative prostitutes from Nairobi – these CTL may confer protection
- Seroprevalence in this cohort is 90-95% and their HIV-1 exposure is among the highest in the world
- Most isolated HIV strains are clade A in Nairobi, although clades C and D are also found – B clade epitopes are often cross-reactive, however stronger responses are frequently observed using A or D clade versions of epitopes
- Clade A version of the epitope: TPGPGIRYPL, clade D version: TPGPGIRYPL

Nef(128–137) Nef(128–137 clade B) TPGPGVRYPL HIV-1-exposed seronegative human(B7,B*8101) [Kaul (2000)]

- 11/16 heavily HIV exposed but persistently seronegative sex-workers in Nairobi had HIV-specific CD8 γ-IFN responses in the cervix – systemic CD8+ T-cell responses tended to be to the same epitopes but at generally lower levels than cervical CD8+ T-cell responses
- Low risk individuals did not have such CD8+ cells
- CD8+ T-cell epitopes DTVLEDINL (3 individuals), SLYNVATL (4 individuals), LSPRTLNAW (3 individuals) and YPLTFGWCF (4 individuals) were most commonly recognized by the HIV-resistant women

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Nef(130–143)	Nef(130–143 LAI)	GPGVRYPLTFGWCY	HIV-1 infection	human(B*57)	[Goulder (1996b)]

- CTL response to this epitope observed in 4 long-term survivors
- Peptide defined on the basis of B*5801 binding motif, yet not cross-restricted except at high concentrations

Nef(130–143)	Nef(121–141)	GPGVRYPLTFGWCY	HIV-1 infection	human(B57)	[Ferrari (2000)]

- One of the 51 HIV-1 epitopes selected by Ferrari *et al.* as good candidate CTL epitopes for vaccines by virtue of being conserved and presented by common HLA alleles

Nef(131–143)	Nef( )	GIRYPLTFGWCFK		human( )	[Jubier-Maurin (1999)]

- 41 new HIV-1 strains describing envelope subtypes of HIV-1 A-H were genetically characterized in the nef region – 34 subtypes were classified in the same subtype in nef and env and 7 of the 41 strains were recombinants
- This region was defined as a CTL epitope region that is conserved among HIV-1 M group subtypes

Nef(132–147)	Nef(132–147 BRU)	GVRYPLTFGWCYKLVP	HIV-1 infection	human(A1, B8)	[Hadida (1992)]

- HIV-1 specific CTLs detected in lymphoid organs

Nef(132–147)	Nef(132–147 BRU)	GVRYPLTFGWCYKLVP	HIV-1 infection	human(B18)	[Culmann (1991)]

- Nef CTL clones from HIV+ donors

Nef(132–147)	Nef(132–147)	GVRYPLTFGWCYKLVP	Vaccine	murine(H-2^d)	[Billaut-Mulot (2001)]

Vaccine: *Vector/type:* DNA with DNA boost, DNA with recombinant protein boost *Strain:* LAI *HIV component:* Gag, Tat, Nef *Stimulatory Agents:* IL-18

- DNA vaccinated BALB/c mice primed and boosted with the multiepitopic vaccine with IL-18 showed lymphoproliferative responses 7 weeks post immunization
- Strong but non-lasting HIV-specific CTL responses were detected by a Cr-release assay and DNA prime/DNA boost was more effective than DNA prime protein boost
- Immunization with either the multiepitopic DNA or with the mixed DNA vaccine induced HIV-1 specific Th1 cytokines (IL-2 and IFN-γ)
- Co-administration of IL-18 increased T-cell responses but decreased anti-HIV antibody levels

Nef(133–148)	Nef(133–148 LAI)	VRYPLTFGWCYKLVPV		human(B57)	[Brander & Walker(1996)]

- P. Goulder, pers. comm.

Nef(134–141)	Nef(138–147 LAI)	RYPLTFGW	HIV-1 infection	human(A*2402)	[Brander & Goulder(2001)]

- C. Brander notes this is an A*2402 epitope

Nef(134–141)	Nef(138–147 SF2)	RYPLTFGW	HIV-1 infection	human(A24)	[Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection

- The breadth and specificity of the response were determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response
- Number of HLA-A24+ individuals that had a CTL response to this epitope broken down by group: 0/0 group 1, 2/3 group 2, and 0/0 group 3

Nef(134–141)	Nef(134–141 LAI)	RYPLTFGW		human(B27)	[Culmann(1998)]

- Optimal peptide defined by titration

Nef(134–143)	Nef(138–147 SF2)	RYPLTFGWCF	HIV-1 infection	human(A*2402)	[Ikeda-Moore (1997)]

- Defined using reverse immunogenetics – 59 HLA-A*2402 binding peptides were predicted by searching for A*2402 anchors in HIV proteins (Tyr at 2, and Phe, Leu or Ile at the C term) – 53 of the 59 peptides bound A*2402
- This peptide induced CTL in 3/4 HIV-1+ people tested
- RYPLTFGWCF bound to A*2402 strongly, the epitope can be processed in a vaccinia construct and presented – two specific CTL clones were obtained

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Nef(134–144)	Nef(134–144 LAI)	RYPLTFGWCYK	HIV-1 infection	human(B18)	[Couillin (1994), Goulder (1997a)]

- Mutational variation in HIV epitopes in individuals with appropriate HLA types can result in evasion of CTL response
- [Goulder (1997a)] is a review of immune escape that summarizes this study

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Nef(134–144)	Nef(134–144)	RYPLTFGWCYK	HIV-1 infection	human(B18)	[Oxenius (2000)]

- Epitope name: RYP. Patients who started therapy at acute HIV-1 infection (three with sustained therapy, two with limited therapy upon early infection) had strong HIV-specific CD4 proliferative responses and were able to maintain a CTL response even with undetectable viral load – three patients that had delayed initiation of HAART had no HIV-specific CD4 proliferative responses and lost their CTL responses when HAART was eventually given and their viral loads became undetectable
- None of the 8 study subjects recognized this epitope but none were HLA B18+

Nef(135–143)	Nef(135–143 LAI)	YPLTFGWCY	*in vitro* stimulation	human(B*0702)	[Lucchiari-Hartz (2000)]

- Five naturally processed MHC class I ligands were identified in Nef in the conserved immunogenic region Nef between 123-152
- All five could be transported by TAP, and 4/5 had N-termini that were major cleavage points for the proteasome, only one had extended precursor fragments
- YPLTFGWCY is the naturally processed ligand for B7, and this epitope is the only one of the five that may require trimming at the N-termini
- YPLTFGWCY is present in low copy number in the cell, possibly due to a predominant proteasomal cleavage site between Y and P

Nef(135–143)	Nef(135–143 LAI)	YPLTFGWCY	HIV-1-exposed seronegative	human(B*1801)	[Brander & Goulder(2001)]

- C. Brander notes this is a B*1801 epitope

Nef(135–143)	Nef(134–142 BRU)	YPLTFGWCY	HIV-1 infection	human(B*5301)	[Mulligan (2001)]

- Epitope N14 from Patient 07107 with HLA genotypes A*3002, A*3201, B*4501, B*5301, Cw*0401, Cw*1202

Nef(135–143)	Nef( )	YPLTFGWCF	HIV-1-exposed seronegative	human(B18)	[Kaul (2000)]

- 11/16 heavily HIV exposed but persistently seronegative sex-workers in Nairobi had HIV-specific CD8 γ-IFN responses in the cervix – systemic CD8+ T-cell responses tended to be to the same epitopes but at generally lower levels than cervical CD8+ T-cell responses
- Low risk individuals did not have such CD8+ cells
- CD8+ T-cell epitopes DTVLEDINL (3 individuals), SLYNVATL (4 individuals), LSPRTLNAW (3 individuals) and YPLTFGWCF (4 individuals) were most commonly recognized by the HIV-resistant women

Nef(135–143)	Nef(135–143 LAI)	YPLTFGWCY	HIV-1-exposed seronegative	human(B18)	[Culmann (1991), Culmann-Penciolelli (1994)]

- Nef CTL clones from HIV+ donors

Nef(135–143)	Nef(135–143 SF2)	YPLTFGWCY	HIV-1 infection	human(B18)	[Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection
- The breadth and specificity of the response were determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response
- Number of HLA-B18+ individuals that had a CTL response to this epitope broken down by group: 0/3 group 1, 1/2 group 2, and 0/0 group 3

Nef(135–143)	Nef(135–143)	YPLTFGWCY	HIV-1-exposed seronegative, HIV-1 infection	human(B18,B49)	[Kaul (2001a)]

- Variants YPLTFGWC[Y/F] are specific for the B/D clades
- ELISPOT was used to study CTL responses to a panel of 54 predefined HIV-1 epitopes in 91 HIV-1-exposed, persistently seronegative (HEPS) and 87 HIV-1-infected female Nairobi sex workers
- Responses in HEPS women tended to be lower, and focused on different epitopes with HLA presenting molecules that have previously been associated with reduced risk of infection, and there was a shift in the response in the HEPS women upon late seroconversion to epitopes recognized by the HIV-1-infected women
- 43/91 HEPS women had CD8+ responses and detection of HIV-1-specific CTL in HEPS women increased with the duration of viral exposure
- Among HLA-B18 women, 1/4 HEPS and 8/9 HIV-1-infected women recognized this epitope, likelihood ratio 5.3, p value 0.04, and HEPS women tended to respond to FRDYVDRF[Y/F]K, while infected women tended to respond to YPLTFGWC[Y/F]
- The dominant response to this HLA allele was to this epitope for the one reactive HEPS case and in all 8/9 HIV-1-infected women

- Differences in epitope specificity were only seen for responses restricted by class I HLA alleles A2, A24, A*6802, B14, and B18, previously shown to be associated with resistance to HIV-1 in this cohort

Nef(135–143)	Nef(139–147 SF2) • Binds HLA-B*3501	YPLTFGWCF	HIV-1 infection	human(B35)	[Shiga (1996)]
Nef(135–143)	Nef( )	YPLTFGWCY	HIV-1-exposed seronegative	human(B49)	[Rowland-Jones (1998a)]

- A CTL response was found in exposed but uninfected prostitutes from Nairobi using previously-defined B clade epitopes that tended to be conserved in A and D clades – such cross-reactivity could protect against both A and D and confer protection in Nairobi where both subtypes are circulating
- The A subtype consensus is identical to the B clade epitope
- The D subtype consensus is YPLTFGWCf

Nef(135–143)	Nef( )	YLPTFGWCY	HIV-1-exposed seronegative	human(B49)	[Rowland-Jones (1998b)]

- HIV-specific CTL were found in exposed seronegative prostitutes from Nairobi – these CTL may confer protection
- Seroprevalence in this cohort is 90-95% and their HIV-1 exposure is among the highest in the world
- Most isolated HIV strains are clade A in Nairobi, although clades C and D are also found – B clade epitopes are often cross-reactive, however stronger responses are frequently observed using A or D clade versions of epitopes
- This epitope is conserved among A and B clade viruses
- The clade D version of the epitope, YPLTFGWCF, was preferentially recognized by CTL

Nef(135–143)	( )	YPLTFGWCY	HIV-1 infection	human(B49)	[Kaul (2001b)]

- This study examines CTL responses in HIV-exposed, persistently seronegative individuals, HEPS, who eventually seroconverted – 11/114 HEPS Nairobi sex workers eventually seroconverted, and for six of these HIV CTL reactive epitopes had been defined while seronegative
- The epidemiological factor associated with seroconversion was stopping sex work and HIV-specific CTL activity declines when HEPS sex workers stop working for a period or retire
- This epitope (YPLTFGWCY/F) was recognized in 1/22 HEPS sex worker controls (ML1668)

Nef(136–145)	Nef(136–145)	PLTFGWCYKL	*in vitro* stimulation	human(A2)	[Wilson (1999b)]

- Dendritic cells are the most potent for priming T-cell responses – DCs can stimulate autologous CTL responses from T-cells cultured from HIV negative donors
- Th1-biasing cytokines IL-12 or IFNα enhance CTL responses *in vitro* whether the epitope is delivered by pulsing from peptide, or expressed from within
- B7 and A2 Nef epitopes were studied and the relative binding affinity of A2 epitopes for A2 was: PLTFGWCYKL greater than VLEWRFDSRL which was much greater than AFHHVAREL

Nef(136–145)	Nef(136–145 LAI)	PLTFGWCYKL		human(A*0201)	[Brander & Goulder(2001)]

- C. Brander notes this is an A*0201 epitope

Nef(136–145)	Nef(136–145 LAI)	PLTFGWCYKL	*in vitro* stimulation	human(A*0201)	[Lucchiari-Hartz (2000)]

- Five naturally processed MHC class I ligands were identified in Nef in the conserved immunogenic region Nef between 123-152
- All five could be transported by TAP, and 4/5 had N-termini that were major cleavage points for the proteasome, only one had extended precursor fragments
- The CTL that recognized PLTFGWCYKL also recognized PLTFGWCYKLV, and both forms of the epitope are naturally processed and both seem to be the direct product of a proteasomal digest, although in low copy number

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Nef(136–145)	Nef(136–145)	PLTFGWCFKL	HIV-1 infection	human(A2)	[Durali (1998)]

- Cross-clade CTL response was studied by determining the CTL activity in seven patients from Bangui, (6 A subtype, and 1 AG recombinant infections) and one A subtype infection from a person living in France originally from Togo, to different antigens expressed in vaccinia
- Pol reactivity: 8/8 had CTL to A subtype, and 7/8 to B subtype, and HIV-2 Pol was not tested
- Gag reactivity: 7/8 reacted with A or B subtype gag, 3/8 with HIV-2 Gag
- Nef reactivity: 7/8 reacted with A subtype, and 5/8 with B subtype, none with HIV-2 Nef
- Env reactivity: 3/8 reacted with A subtype, 1/8 with B subtype, none with HIV-2 Env
- Patient B18 had the greatest breadth and diversity of response, and recognized Gag SLYNTVATL and Nef PLTFGWCFKL

Nef(136–145)	Nef(157–166)	PLTFGWCFKL	Vaccine	human(A2)	[Woodberry (1999)]

Vaccine: *Vector/type:* DNA prime with vaccinia boost *HIV component:* polyepitope

- A polyepitope vaccine was generated in a vaccinia construct that contiguously encoded seven epitopes, all presented by HLA A-2
- HHD mice have a transgene of HLA A2 linked to the transmembrane and cytotoxic domains of H-2D^d – this transgene is the only MHC molecule expressed in the mice
- CTL responses to Gag (77-85) SLYNTVATL, Pol (476-484) ILKEPVHGV, gp120 (120-128) KLTPLCVTL, and Nef (190-198) AFHHVAREL were observed in HIV polytope HHD-vaccinated mice, and these responses were enhanced with vaccinia boost
- No CTL immune responses were generated against HLA A2-restricted HIV epitopes Nef 157-166 (PLTFGWCYKL), Pol 346-354 (VIYQYMDDL), and Nef 180-189 (VLEWRFDSRL)
- Sixteen HLA A2+ patients were tested for their ability to make CTL responses by peptide restimulation in culture with the epitopes selected for inclusion in the polytope – one individual recognized all seven of these epitopes; 7 patients had CTL cultures able to recognize at least one of the epitopes, and 6 of those 7 recognized more than one epitope, but they were not able to test all peptides for all patients; many patients only had three peptides tested
- PLTFGWCFKL was recognized by 1 of the HLA-A2 patients

Nef(136–145)	Nef(135–144 93TH253 CRF01)	PLTFGWCYKL	HIV-1 infection	human(A2)	[Bond (2001)]

- HLA-A11 CRF01 (called subtype E in Bond *et al*.) epitopes were identified that stimulated CTL from HIV+ female sex workers (FSW) from Northern Thailand, of whom more than half were HLA-A11 positive so the study concentrated on A11 epitopes, although E clade versions of previously defined B-clade A2 and A24 epitopes were also tested
- 0/4 tested FSWs recognized the E clade version of this epitope PLCFGWCFKL, which differs from the previously defined B clade version by two amino acids, PLTFGWCYKL
- This epitope was only conserved in CRF01 (subtype E) and subtype B

Nef(136–145) Nef(136–145) PLTFGWCYKL HIV-1 infection human(A2) [Day (2001)]

- The CTL response to optimally defined CTL epitopes restricted by HLA class I A and B alleles in individuals who co-expressed HLA A2, A3, and B7 was studied in eight HIV-1-infected subjects, two with acute infection, five with chronic, and one long-term non-progressor (LTNP)
- Two to 17 epitopes were recognized in a given individual, A2-restricted CTL response tended to be narrow and never dominated the response, and 25/27 epitopes were targeted by at least one person

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Nef(136–145) Nef(158–166) LTFGWCFKL HIV-1 infection human(A2 supertype) [Propato (2001)]

- Long-term non-progressors (LTNPs) had strong memory resting CD8+ T-cell responses against the majority of epitopes tested (18 for the A2 supertype, 16 for the A3 supertype), while the effector cells of long-term non-progressors recognized far fewer epitopes
- Progressors had memory resting CD8+ T-cells that recognized far fewer epitopes than LTNPs
- A positive correlation between effector CD8+ T-cells and plasma viremia and a negative correlation between CD8+ effector T-cells and CD4+ T-cells was observed, which may contribute to the inability of LTNPs to clear virus
- This epitope can bind five HLA-A2 supertypes alleles (A*0201, A*0202, A*0203, A*0206 and A*6802)

Nef(136–146) Nef(136–146 LAI) PLTFGWCYKLV *in vitro* stimulation human(A*0201) [Lucchiari-Hartz (2000)]

- Five naturally processed MHC class I ligands were identified in Nef in the conserved immunogenic region Nef between 123-152
- All five could be transported by TAP, and 4/5 had N-termini that were major cleavage points for the proteasome, only one had extended precursor fragments
- The CTL that recognized PLTFGWCYKL also recognized PLTFGWCYKLV, and both forms of the epitope are naturally processed and both seem to be the direct product of a proteasomal digest, although in low copy number

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Nef(136–146) Nef(158–167) LTFGWCFKLV HIV-1 infection human(A2 supertype) [Propato (2001)]

- Long-term non-progressors (LTNPs) had strong memory resting CD8+ T-cell responses against the majority of epitopes tested (18 for the A2 supertype, 16 for the A3 supertype), while the effector cells of long-term non-progressors recognized far fewer epitopes
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- This epitope can bind five HLA-A2 supertype alleles (A*0201, A*0202, A*0203, A*0206 and A*6802)
- Tetramer staining with A2, β2-microglobulin, and either SLYNTVATL, KLVGKLNWA, or LTFGWCFKL revealed that tetramers detected more HIV-specific cells in LTNP than in progressors, activated effector cells were the minority population, and ELISPOT correlated better with the effector cell subpopulation than the total tetramer stained population

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Nef(137–146)	Nef( )	LTFGWCFKLV	HIV-1 infection	human(A2)	[Altfeld (2001d)]

- Epitope name: Nef-221a. HIV was scanned for all peptides which carried the A2-supermotif pattern conserved in more than 50% of B clade sequences – 233 peptides met this criteria, and 30 of these bound to HLA-A*0201 – 20/30 bound to at least 3/5 of HLA-A2 supertype alleles tested
- Three additional previously described HLA-A2 epitopes were added to the set of 20, and 18/22 chronically infected HLA-A2 individuals had CTL that recognized at least one of the 23 peptides (median of 2 and maximum of 6), while 6/12 acutely infected individuals recognized at least 1 (median of 1 and maximum of 2)
- 1/22 individuals with chronic HIV-1 infection recognized this epitope in ELISPOT
- 2/12 acutely infected individuals recognized this epitope
- LTFGWCFKLV binds to five HLA-A2 supertype alleles:A*0203, A*0201 (highest affinity), A*0206, A*6802 and A*0202

Nef(162–181)	Nef(161–180)	TSLLHPVSLHGMDDP-EREVL	*in vitro* stimulation	human( )	[Lieberman (1995)]

- HIV-specific CTL lines developed by *ex vivo* stimulation with peptide

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Nef(162–181)	Nef(161–180 SF2)	TSLLHPVSLHGMDDP-EREVL	HIV-1 infection	human( )	[Lieberman (1997a)]

- Of 25 patients, most had CTL specific for more than one HIV-1 protein
- Eleven subjects had CTL that could recognize vaccinia-expressed LAI Nef
- One of these 11 had CTL response to this peptide

Nef(162–181)	Nef(101–120 SF2)	TSLLHPVSLHGMDDP-EREVL	HIV-1 infection	human( )	[Lieberman (1997b)]

- CTL expanded *ex vivo* were later infused into HIV-1 infected patients

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Nef(162–181)	Nef(161–180 SF2)	TSLLHPVSLHGMDDP-EREVL	HIV-1 infection	human( )	[Lieberman (1997a)]

- Of 25 patients, most had CTL specific for more than one HIV-1 protein
- Eleven subjects had CTL that could recognize vaccinia-expressed LAI Nef
- One of these 11 had CTL response to this peptide

Nef(166–177)	Nef(160–179 SF2)	HPVSLHGMDDPE	HIV-1 infection	human(B35)	[Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection
- The breadth and specificity of the response were determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response

- Number of HLA-B35+ individuals that had a CTL response to this epitope broken down by group: 1/2 group 1, 0/2 group 2, and 0/1 group 3

Nef(172–191) Nef(171–190 SF2) GMDDPEREVLEWRFD-SRLAF HIV-1 infection human( ) [Lieberman (1997a)]

- Of 25 patients, most had CTL specific for more than one HIV-1 protein
- Eleven subjects had CTL that could recognize vaccinia-expressed LAI Nef
- One of these 11 had CTL response to this peptide
- The responding subject was HLA-A2, B21

Nef(175–184) Nef(175–184) DPEKEVLQWK HIV-1 infection human(B7) [Jin (2000b)]

- This a B7 epitope, a subdominant CTL response, was defined by an un-conventional approach used to predict epitopes in an HLA B7+ long-term non-progressor
- Three additional sub-dominant HLA B7 epitopes were defined using EpiMatrix, a non-anchor based strategy for defining potential epitopes, which highlighted 2078 possible epitopes in the autologous HIV-1 derived from the study subject, followed by B7 anchor residue prediction which narrowed the set to 55 peptides, three of which could serve as functional CTL epitopes

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Nef(179–187) Nef( ) AFHHVAREL HIV-1 infection human(A*0201) [Altfeld (2001d)]

- Epitope name: Nef AL9. HIV was scanned for all peptides which carried the A2-supermotif pattern conserved in more than 50% of B clade sequences – 233 peptides met this criteria, and 30 of these bound to HLA-A*0201 – 20/30 bound to at least 3/5 of HLA-A2 supertype alleles tested
- Three additional previously described HLA-A2 epitopes were added to the set of 20, including Nef AL9, and 18/22 chronically infected HLA-A2 individuals had CTL that recognized at least one of the 23 peptides (median of 2 and maximum of 6), while 6/12 acutely infected individuals recognized at least 1 (median of 1 and maximum of 2)
- RT VL9 was not recognized by any of the 22 HLA-A2 patients with chronic HIV-1 infection or the 13 HLA-A2 patients with acute HIV-1 infection included in this study

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Nef(180–189) Nef(180–189 LAI) VLEWRFDSRL HIV-1 infection human(A*0201) [Haas (1996), Haas (1997)]

- There was a high degree of variation in three CTL epitopes in Nef in four slow and non-progressors, and variant specific CTLs arose over time to eliminate variants, indicating immune selection
- Noted in Brander *et al*., 1999 this database, to be A*0201

Nef(180–189) Nef(180–189 LAI) VLEWRFDSRL human(A*0201) [Brander & Goulder(2001)]

- C. Brander notes this is an A*0201 epitope

Nef(180–189) Nef(180–189) VLEWRFDSRL *in vitro* stimulation human(A2) [Wilson (1999b)]

- Dendritic cells are the most potent for priming T-cell responses – DCs can stimulate autologous CTL responses from T-cells cultured from HIV negative donors

- Th1-biasing cytokines IL-12 or IFNα enhance CTL responses *in vitro* whether the epitope is delivered by pulsing from peptide, or expressed from within
- B7 and A2 Nef epitopes were studied and the relative binding affinity of A2 epitopes for A2 was: PLTFGWCYKL greater than VLEWRFDSRL which was much greater than AFHHVAREL

Nef(180–189) Nef(180–189) VLEWRFDSRL Vaccine human(A2) [Woodberry (1999)]

Vaccine: *Vector/type:* DNA prime with vaccinia boost *HIV component:* polyepitope

- A polyepitope vaccine was generated in a vaccinia construct that contiguously encoded seven epitopes, all presented by HLA A-2
- HHD mice have a transgene of HLA A2 linked to the transmembrane and cytotoxic domains of H-2D^d – this transgene is the only MHC molecule expressed in the mice
- CTL responses to Gag (77-85) SLYNTVATL, Pol (476-484) ILKEPVHGV, gp120 (120-128) KLTPLCVTL, and Nef (190-198) AFHHVAREL were observed in HIV polytope HHD-vaccinated mice, and these responses were enhanced with vaccinia boost
- No CTL immune responses were generated against HLA A2-restricted HIV epitopes Nef 157-166 (PLTFGWCYKL), Pol 346-354 (VIYQYMDDL), and Nef 180-189 (VLEWRFDSRL)
- Sixteen HLA A2+ patients were tested for their ability to make CTL responses by peptide restimulation in culture with the epitopes selected for inclusion in the polytope – one individual recognized all seven of these epitopes; 7 patients had CTL cultures able to recognize at least one of the epitopes, and 6 of those 7 recognized more than one epitope, but they were not able to test all peptides for all patients; many patients only had three peptides tested
- VLEWRFDSRL was recognized by 2 of the HLA-A2 patients

Nef(180–189) Nef(180–189 LAI) VLEWRFDSRL HIV-1 infection human(A2) [Mollet (2000)]

- Epitope name: N3. A panel of 16 epitopes covering 15 class I alleles was tested in 14 HIV+ patients from an unselected Caucasian population treated with HAART, using CD8+ cell IFNγ production to measure responses
- In general, during the first month of treatment viral load decreased and frequencies of HIV-specific CTL tripled and broadened – eight new HIV specificities that were not previously detectable were newly detected, as were CMV specific CD8+ PBL – but with continued viral suppression, HIV-specific responses diminished
- Viral rebounds gave different patterns of response: increases or decreases in pre-existing response, new specificities, or no change

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Nef(180–189) Nef(179–188 93TH253 CRF01) VLEWRFDSRL HIV-1 infection human(A2) [Bond (2001)]

- HLA-A11 CRF01 (called subtype E in Bond *et al*.) epitopes were identified that stimulated CTL from HIV+ female sex workers (FSW) from Northern Thailand, of whom more than half were HLA-A11 positive so the study concentrated on A11 epitopes, although E clade versions of previously defined B-clade A2 and A24 epitopes were also tested
- 0/4 tested FSWs recognized the E clade version of this epitope VLIWKFDSAL, which differs from the previously defined B clade version by three amino acids, VLEWRFDSRL
- This epitope was only conserved in CRF01 (subtype E), and identities were rare

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Nef(180–189)	Nef(180–189)	VLEWRFDSRL	HIV-1 infection	human(A2)	[Day (2001)]

- The CTL response to optimally defined CTL epitopes restricted by HLA class I A and B alleles in individuals who co-expressed HLA A2, A3, and B7 was studied in eight HIV-1-infected subjects, two with acute infection, five with chronic, and one long-term non-progressor (LTNP)
- Two to 17 epitopes were recognized in a given individual, A2-restricted CTL response tended to be narrow and never dominated the response, and 25/27 epitopes were targeted by at least one person

Nef(182–198)	Nef(182–198 BRU)	EWRFDSRLAFHHVAR-EL	HIV-1 infection	human(A1, B8)	[Hadida (1992)]

- HIV-1 specific CTLs detected in lymphoid organs of HIV-1 infected patients

Nef(182–198)	Nef(182–198 LAI)	EWRFDSRLAFHHVAR-EL	HIV-1 infection	human(A2, A25(10))	[Hadida (1995)]

- The C-terminal region of Nef (182-205) contains multiple CTL epitopes with 5 distinct HLA restrictions

Nef(182–198)	Nef(182–198 BRU)	EWRFDSRLAFHHVAR-EL	HIV-1 infection	human(A25)	[Cheynier (1992)]

- CTL isolated in children born to HIV-1 positive mothers

Nef(182–198)	Nef(182–198 LAI)	EWRFDSRLAFHHVAR-EL	HIV-1 infection	human(B35)	[Hadida (1995)]

- The C-terminal region of Nef (182-205) contains multiple CTL epitopes with 5 distinct HLA restrictions

Nef(182–198)	Nef(182–198 LAI)	EWRFDSRLAFHHVAR-EL	Vaccine	murine(H-2^d)	[Van der Ryst (1998)]

Vaccine: *Vector/type:* Mengo virus, vaccinia *Strain:* LAI *HIV component:* Nef

- Macaca mulatta did not have a detectable response to Rec Mengo virus-HIV-1 Nef 65-206 vaccine
- BALB/c mice had a weak response to this epitope in the Mengo virus construct – in contrast, HIV-1 Nef induces a strong CTL response in mice when presented in a vaccinia background

Nef(182–201)	Nef(191–205 SF2)	EWRFDSRLAFHHVAR-ELHPE	HIV-1 infection	human( )	[Lieberman (1997a)]

- Of 25 patients, most had CTL specific for more than one HIV-1 protein
- Eleven subjects had CTL that could recognize vaccinia-expressed LAI Nef
- One of these 11 had CTL response to this peptide
- The responding subject was HLA-A2, B21

Nef(182–205)	Nef(182–205 LAI)	EWRFDSRLAFHHVAR-ELHPEYFKN	Vaccine	human( )	[Gahery-Segard (2000)]

Vaccine: *Vector/type:* lipopeptide *HIV component:* six peptides

- Anti-HIV lipopeptide vaccine consisting of six long amino acid peptides derived from Nef, Gag and Env HIV-1 proteins modified by a palmitoyl chain was administered in a phase I trial
- A CD4+ T-cell proliferative response to at least one of the six peptides was observed in 9/10 vaccinees – 4/10 reacted to this Nef peptide
- 9/12 tested mounted a CTL response to at least one of the six peptides; each of the six peptides elicited a CTL response in at least one individual
- None of the 12 tested had an IgG response to this peptide

Nef(183–191)	Nef(182–190 BRU)	WRFDSRLAF	HIV-1 infection	human(B*1503)	[Mulligan (2001)]
• Epitope N18 from Patient 11113 with HLA genotypes A*2904, A*3002, B*1503, B*5802, Cw*0202, Cw*0602					
Nef(186–193)	Nef(186–193 LAI)	DSRLAFHH	HIV-1 infection	human(B35)	[Hadida (1995)]
• The C-terminal region of Nef (182-205) contains multiple CTL epitopes with 5 distinct HLA restrictions					
Nef(186–194)	Nef(186–194)	DSRLAFHHM	HIV-1-exposed seronegative, HIV-1 infection	human(A24)	[Kaul (2001a)]
• ELISPOT was used to study CTL responses to a panel of 54 predefined HIV-1 epitopes in 91 HIV-1-exposed, persistently seronegative (HEPS) and 87 HIV-1-infected female Nairobi sex workers					
Nef(186–194)	Nef(186–194 BRU)	DSRLAFHHV		human(B51)	[Connan (1994)]
• Resulted in the assembly of HLA-B51					
Nef(188–196)	Nef(188–196 LAI)	RLAFHHVAR	HIV-1 infection	human(B52)	[Hadida (1995)]
• The C-terminal region of Nef (182-205) contains multiple CTL epitopes with 5 distinct HLA restrictions					
Nef(188–201)	Nef(188–201 LAI)	RLAFHHVARELHPE	HIV-1 infection	human(B35 or C4)	[Buseyne (1993a)]
• Vertical transmission of HIV ranges from 13% to 39% • Primary assays showed cytotoxic activity against at least one HIV protein was detected in 70% of infected children • Epitopes recognized in five children were mapped using synthetic peptides and secondary cultures • Patient EM13, who had a CTL response to three epitopes in Nef, was infected via blood transfusion after birth and went from CDC stage P2A to P2E during the study					
Nef(190–198)	( )	ALKHRAYEL	HIV-1 infection	human( )	[Kaul (2001b)]
• This study examines CTL responses in HIV-exposed, persistently seronegative individuals, HEPS, who eventually seroconverted – 11/114 HEPS Nairobi sex workers eventually seroconverted, and for six of these HIV CTL reactive epitopes had been defined while seronegative • The epidemiological factor associated with seroconversion was stopping sex work and HIV-specific CTL activity declines when HEPS sex workers stop working for a period or retire • This epitope was in 1/22 HEPS controls, ML1749					

Nef(190–198) Nef(190–198 LAI) AFHHVAREL HIV-1-exposed seronegative human(A2) [Rowland-Jones (1998a)]

- CTL recognition reported in the context of HLA-B52 and A2.1, A2.2 and A2.4
- A CTL response was found in exposed but uninfected prostitutes from Nairobi using previously-defined B clade epitopes that tended to be conserved in A and D clades – such cross-reactivity could protect against both A and D and confer protection in Nairobi where both subtypes are circulating
- The A subtype consensus is ALKHRAYEL
- The D subtype consensus is AfEHKAREm
- [Hunziker1998] maintains that HLA-A2 does not present this epitope contrary to an earlier report [Hadida (1995)], (also see [Brander (1998)]) – despite the position of Hunziker *et al.*, Rowland-Jones and colleagues are confident that this epitope in its A clade form is presented by HLA-A*0201 and A*0202, and it is one of the most common responses seen in both seropositive and exposed-uninfected donors from Nairobi (Rupert Kaul, Pers. Comm.)

Nef(190–198) Nef(190–198) AFHHVAREL *in vitro* stimulation human(A2) [Wilson (1999b)]

- Dendritic cells are the most potent for priming T-cell responses – DCs can stimulate autologous CTL responses from T-cells cultured from HIV negative donors
- Th1-biasing cytokines IL-12 or IFNα enhance CTL responses *in vitro* whether the epitope is delivered by pulsing from peptide, or expressed from within
- B7 and A2 Nef epitopes were studied and the relative binding affinity of A2 epitopes for A2 was: PLTFGWCYKL greater than VLEWRFDSRL which was much greater than AFHHVAREL

Nef(190–198) Nef(190–198) AFHHVAREL Vaccine human(A2) [Woodberry (1999)]

Vaccine: *Vector/type:* vaccinia *HIV component:* polyepitope

- A polyepitope vaccine was generated in a vaccinia construct that contiguously encoded seven epitopes, all presented by HLA A-2
- HHD mice have a transgene of HLA A2 linked to the transmembrane and cytotoxic domains of H-2D^d – this transgene is the only MHC molecule expressed in the mice
- CTL responses to Gag (77-85) SLYNTVATL, Pol (476-484) ILKEPVHGV, gp120 (120-128) KLTPLCVTL, and Nef (190-198) AFHHVAREL were observed in HIV polytope HHD-vaccinated mice, and these responses were enhanced with vaccinia boost
- No CTL immune responses were generated against HLA A2-restricted HIV epitopes Nef 157-166 (PLTFGWCYKL), Pol 346-354 (VIYQYMDDL), and Nef 180-189 (VLEWRFDSRL)
- Sixteen HLA A2+ patients were tested for their ability to make CTL responses by peptide restimulation in culture with the epitopes selected for inclusion in the polytope – one individual recognized all seven of these epitopes; 7 patients had CTL cultures able to recognize at least one of the epitopes, and 6 of those 7 recognized more than one epitope, but they were not able to test all peptides for all patients; many patients only had three peptides tested
- AFHHVAREL was recognized by 2 of the patients

Nef(190–198) Nef(190–198 SF2) AFHHVAREL HIV-1 infection human(A2) [Altfeld (2001c)]

- Therapy provided during acute infection resulted in a narrower CTL response, stronger T help response, and a less diverse viral population than was seen in individuals treated during chronic infection

- The breadth and specificity of the response were determined using ELISPOT by studying 19 individuals with pre-seroconversion therapy (Group 1), 11 individuals with primary infection but post-seroconversion therapy (Group 2), and 10 individuals who responded to HAART given during chronic infection (Group 3), using 259 overlapping peptides spanning p17, p24, RT, gp41, gp120 and Nef
- Previously described and newly-defined optimal epitopes were tested for CTL response
- Number of HLA-A2+ individuals that had a CTL response to this epitope broken down by group: 0/10 group 1, 1/6 group 2, and 0/4 group 3

Nef(190–198)	Nef(190–198)	ALKHRAYEL	HIV-1-exposed seronegative, HIV-1 infection	human(A2)	[Kaul (2001a)]

- Variants ALKHRAYEL and AFHHVAREL are A/B clade specific
- ELISPOT was used to study CTL responses to a panel of 54 predefined HIV-1 epitopes in 91 HIV-1-exposed, persistently seronegative (HEPS) and 87 HIV-1-infected female Nairobi sex workers

Nef(190–198)	Nef( )	AFHHVAREL	HIV-1-exposed seronegative	human(A2, A*0202, A*0201)	[Rowland-Jones (1998b)]

- HIV-specific CTL were found in exposed seronegative prostitutes from Nairobi – these CTL may confer protection
- Seroprevalence in this cohort is 90-95% and their HIV-1 exposure is among the highest in the world
- Most isolated HIV strains are clade A in Nairobi, although clades C and D are also found – B clade epitopes are often cross-reactive, however stronger responses are frequently observed using A or D clade versions of epitopes
- Clade A version of the epitope: ALKHRAYEL, clade D epitope: AFEHKAREM
- This epitope was recognized by two different exposed and uninfected prostitutes

Nef(190–198)	Nef(190–198 LAI)	AFHHVAREK	HIV-1 infection	human(A3)	[Hadida (1995)]

- Naturally-occurring L to K anchor substitution abrogates A2 binding, but permits HLA-A3 binding

Nef(192–206)	Nef(192–206 BRU)	HHVARELHPEYFKNC	HIV-1 infection	human(A1)	[Hadida (1992)]

- HIV-1 specific CTLs detected in lymphoid organs of HIV-1 infected patients

Nef( )	Nef( )		HIV-1 infection	human( )	[Wasik (2000)]

- HIV+ infants that progressed rapidly to AIDS had lower Th1 responses and decreased production of β-chemokines and IL-2 relative to other HIV+ infants
- No HIV+ infants had no demonstrable CTL at birth, but Th1 responses accompanied by CTL responses developed in children with slowly progressive disease, and not in rapid progressors
- CTLp frequencies were determined by limiting dilution using autologous B cells infected with vaccinia/HIV constructs

Nef( )	Nef( )		HIV-1 infection	human( )	[De Maria (1997)]

- CD3+ cells that also carry a natural killer cell receptor (NKR+) can exhibit down regulation of T-cell function
- Anti-NKR IgM MAb masked this inhibitory function and increased HIV-1 specific CTL activity in phytohemagglutinin-activated PBMC cultured in the presence of IL-2 from 3/5 patients, and in one other case anti-NKR MAb brought HIV-1 specific CTL activity to detectable levels

Nef( ) Nef( ) HIV-1 infection human( ) [Lubaki (1999)]

- Three strategies were used to analyze CTL activity: area under the net HIV-specific lysis curve (ACU), linear regression (LR) of net specific lysis, and the standard method, lytic units (LU20)
- A correlation between low HIV plasma viral load and increased levels of HIV-specific Gag and Nef CTL activity was observed using ACU and LR, but not LU20

Nef( ) Nef( ) Vaccine human( ) [Gorse (1999)]

Vaccine: *Vector/type:* canarypox prime with rgp120 boost *Strain:* LAI and SF2 *HIV component:* Env, Gag, Pro, Nef, Pro

- The vaccine used was rec canarypox expressing HIV-1 env, gag, pol, nef and protease (vCP300) with or without administration of HIV-1 SF-2 rgp120
- In vitro inducible CTL activity against HIV-1 Env, Gag, Pol, and Nef antigens was observed in 79% (15/19) of vaccine recipients
- The combination of vCP300 and vP1291 together resulted in an overall increase in CTL induction and detection sensitivity

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Nef( ) Nef( ) HIV-1 infection human( ) [Gamberg (1999)]

- 13/13 subjects with advanced HIV infections showed CD8 T-cell proliferation and differentiation of CTL *in vitro*, and six individuals showed HIV-specific responses to Gag, Pol, Env or Nef antigens
- Data suggests that the functional and genetic integrity of the CD8 T-cell repertoire (TCR betaV gene intrafamily genetic diversity) remains intact through advanced HIV infection, although HIV-specific CTL activity decreases

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Nef( ) Nef( ) Vaccine human( ) [Calarota (1999)]

Vaccine: *Vector/type:* DNA *HIV component:* Nef, Rev Tat

- 9/9 HIV-1+ subjects were given one of three DNA vaccinations for nef, rev or tat, and novel proliferative and CTL responses were generated
- The nef DNA immunization induced the highest and most consistent CTLp activity, IFN-γ production, and IL-6 and IgG responses
- Highly active antiretroviral treatment (HAART) did not induce new HIV-specific CTL responses but reduced viral load, while DNA vaccination induced new immune responses but did not reduce viral load – thus this is a potentially complementary and promising combination

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Nef( ) Nef( ) HIV-1 infection human( ) [Buseyne (1998a)]

- This study showed a correlation between strong CTL memory and breadth of response in 7-12 month old infants and: remaining AIDS-free for the first year of life, higher absolute CD4 and CD8 cells, and lower viral load

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Nef( ) Nef( ) HIV-1 infection human( ) [Buseyne (1998b)]

- In infants with positive CTL responses, most responses showed cross-clade reactivity with somewhat diminished recognition of epitopes from different subtypes

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Nef( ) Nef( ) Vaccine human( ) [Evans (1999)]

Vaccine: *Vector/type:* canarypox *HIV component:* gp120, gp41, Gag, Pro, Nef, RT

- A Canarypox vaccine expressing gp120, gp41, Gag, Protease, Nef and Pol CTL epitopes gave rise to CTL that could be detected in 61% of the volunteers – responses to Gag, Env, Nef and Pol were detected 3-6 months after the last vaccination

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Nef( ) Nef( ) HIV-1 infection human( ) [da Silva & Hughes(1998)]

- CTL dense regions of Nef tend to lie in conserved domains with low non-synonymous substitution per site – authors consider that this may be due to a host adaptation to infection that focuses the CTL response to be directed against conserved functional domains [da Silva & Hughes(1998)]

Nef( ) Nef( ) HIV-1 infection human( ) [Legrand (1997)]

- Seventeen recently infected patients were tested for CTL response to HIV proteins Env, Gag, Pol, Rev, Nef, Vif and Tat
- An early response (within a month following PI) was noted in 87% of the subjects to Gag, 75% to Env, and 50% to Nef
- Early responses to Pol, Rev, Vif and Tat were rare

Nef( ) Nef( ) HIV-1 infection human( ) [Zerhouni (1997)]

- CTL responses to Env, Gag, Nef and RT were tested at various phases of disease progression – 10 asymptomatic patients generally had CTL responses to all proteins, 10 ARC patients responded well to all proteins except Nef, and AIDS patients had few responses to any proteins

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Nef( ) Nef( ) HIV-1 infection ( ) [Kuiken (1999)]

- A correlation between conserved regions of Nef and CTL epitope density was also noted in [Kuiken (1999)]. The authors suggest that this may be due to biological reasons such as the one described above [da Silva & Hughes(1998)], or due to epitope processing, or may possibly be an artifact of experimental strategy for epitope definition such that conserved epitopes would tend to be identified because they would be more likely to be cross-reactive with the test reagents
- Both p17 and Nef show a correlation between epitope density and conserved regions in the protein – in contrast, p24 is a more conserved protein and known epitopes are evenly distributed across p24

Nef( ) Nef( ) HIV-1 infection human( ) [Aladdin (1999)]

- In vitro measurements of CTL-activity by Cr release assay in bulk culture showed no correlation between CTL-activity (gp120, Gag, Pol and Nef) and disease progression as measured by viral load, CD4 and time to death

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Nef( ) Nef( ) HIV-1 infection human( ) [Jin (1998a)]

- CTL precursor frequencies were determined in HIV-1 infected pregnant women, and higher CTLp frequencies to Pol and SF2 Nef, but not IIIB Nef, were found in non-transmitting mothers than in transmitting mothers – Nef CTL responses have been found in uninfected infants born to HIV+ women (Lazuriaga95);

Nef( ) ( ) human( ) [Novitsky (2001)]

- This study provides a survey of CTL responses and full length HIV-1 genome sequences from a C subtype infected Botswanan cohort
- 37/45 subjects (82%) demonstrated Nef specific ELISPOT CTL responses of more than 100 SFC/106 PBMC
- Two Nef-immunodominant regions were identified, one spanned amino acid positions 67 to 96 using HXB2 numbering system while the second corresponded to amino acid positions 122 to 141

- While there was some subtype B and C cross-reactivity, there was greater breadth and intensity of response if the CTL from HIV-1-infected individuals was probed with ELISPOT using peptides derived from the same subtype (a median of three Nef epitopes recognized within subtype C compared with one Nef epitope recognized from subtype B peptides, and ELISPOT results with a median of 763 SFC/106 PBMC among responses to HIV-1 C, versus a median of 318 SFC/106 PBMC among responses to HIV-1 B

Nef( ) Nef( ) HIV-1 infection human( ) [Cao (2000)]

- HIV-1 subtypes A and D dominate the Ugandan epidemic, and a vaccine trial using B clade antigen is underway – this study addresses relative levels of cross-reactive CTL responses in HIV infected Ugandans to A, D, and B clade recombinant vaccinia viruses expressing Gag, Env, Pol, RT or Nef from HIV-1 clades A, B, and D
- Proteins corresponding to the subtype of the infecting strains tended to trigger higher levels of CTL response measured by percent-specific lysis, but there was extensive inter-subtype cross-reactivity with B clade proteins and the co-circulating subtype

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Nef( ) Nef( ) HIV-1 infection, Vaccine human( ) [Calarota & Wahren(2001)]

Vaccine: *Vector/type:* DNA *HIV component:* Nef, Rev, Tat *Stimulatory Agents:* CpG motifs

- This review discusses the cellular immune response, and comments on the stimulatory role of CpG motifs and how HIV-1 DNA vaccines can boost the CTL and Th proliferative responses in asympotomatic HIV+ individuals

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Nef( ) Nef( ) HIV-1 infection human(A*0201, Cw*08) [Shacklett (2000)]

- HIV-1 specific, MHC class I-restricted CTL killing was detected in duodenal and rectal gut associated lymphoid tissue (GALT) sites from three infected individuals – the distribution of class I restricted CTL was different in the peripheral blood samples and GALT samples

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Nef( ) Nef( ) Vaccine murine(H-2D^d) [Collings (1999)]

Vaccine: *Vector/type:* DNA *Strain:* BRU *HIV component:* nef

- A comparison of DNA vaccination with HIV-1 Nef expression vectors pBN-CMV-NEF and pBN-RSV-NEF (self-replicating), pCGE2-NEF (non-replicating).
- CTL immune responses were detected using all three expression vectors, while a humoral immune response to Nef was only observed in the self-replicating expression vectors; possibly antibody responses require higher levels of protein expression

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Nef( ) Nef(139–147 HXB3) LTFGWCFKL Vaccine murine(HLA-A201 transgenic) [Sandberg (2000)]

Vaccine: *Vector/type:* DNA, peptide *Strain:* HXB3 *HIV component:* Nef *Stimulatory Agents:* Freund's adjuvant

- Ten Nef 9-mer peptides were predicted to have a strong binding affinity with HLA-A*0201 – of these, four did bind strongly by a T2 class I stabilization assay, several others bound weakly
- A CTL immune response to only 3/10 peptides was detected by a 51Cr-release assay after immunization of HLA-A201 transgenic mice with either nef DNA under the control of a CMV promotor, coated on gold particles delivered to abdominal skin by gene gun – LTFGWCFKL did not elicit a CTL response

- LTFGWCFKL was also tested by subcutaneous injection of Nef peptides in Freund's adjuvant, because it bound strongly to HLA-A*0201, and the peptide vaccination did elicit a response
- The lack of response to the nef DNA vaccine and the response to the peptide suggests LTFGWCFKL may not be processed

Nef( )	Nef( )	SIV Nef and Env CTL epitopes	SIV infection	Rhesus macaque(Mamu-A*11, -B*03, -B*04, and -B*17)	[Dzuris (2000)]

- Cell binding assays for Mamu molecules were employed to describe the peptide binding motifs for Mamu-A*11, -B*03, -B*03, -B*04, and -B*17 CTL epitopes – a similarity for Mamu-A*11 and -B*03 and human HLA-B*44 and -B*27, respectively, was observed – all epitopes studied were SIV epitopes, so not specifically listed here